Natural and Sexual Selection at Work: the Structure and Function of Fallow Deer (*Dama dama*) Vocalisations

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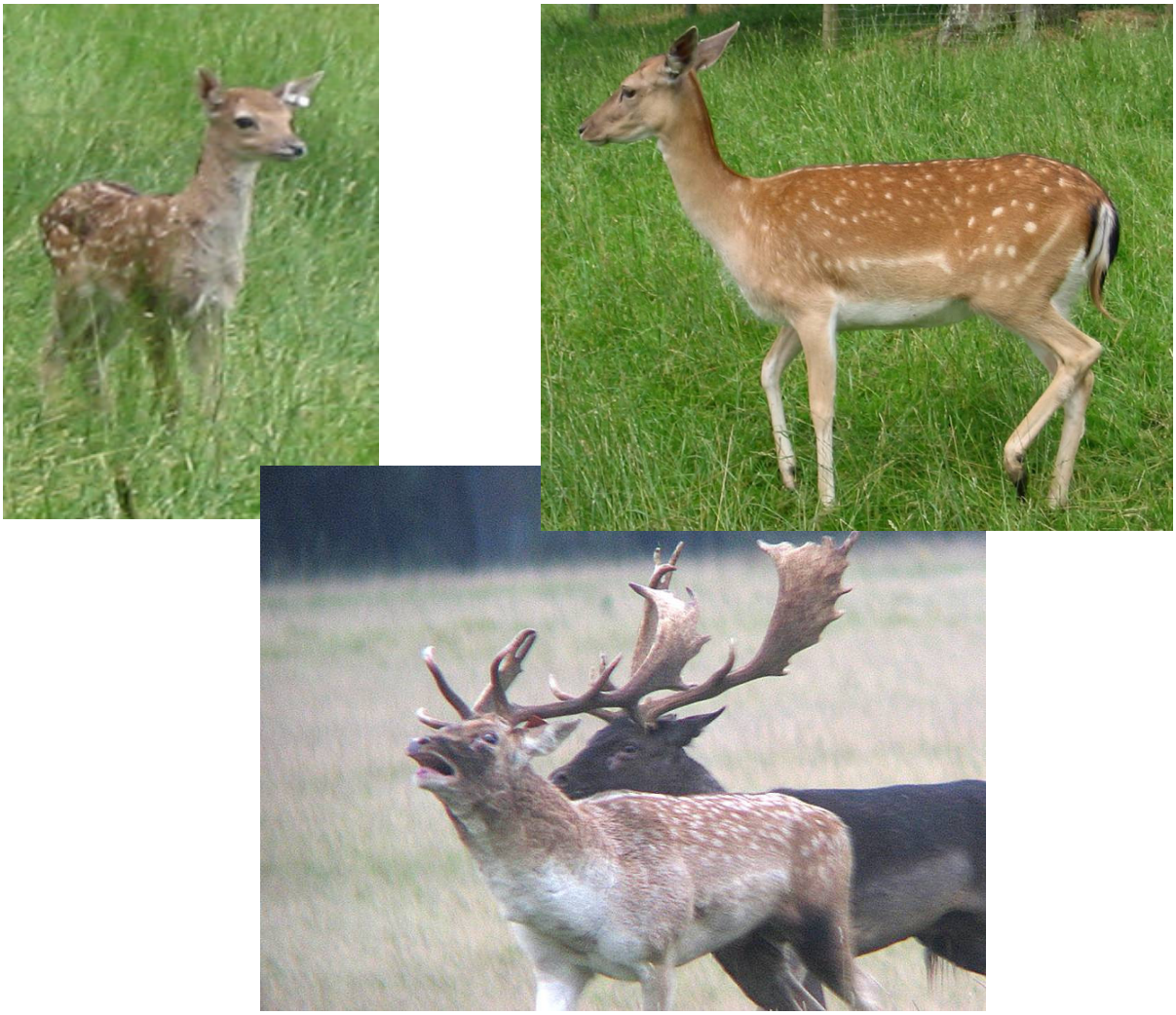

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SUMMARY

Vocal communication in mammals has evolved in response to both natural and sexual selection. During this process, the structure of the vocal apparatus and the mechanism of sound production may place strong constraints on the evolution of acoustic signals. It is therefore important to identify the sources of acoustic variation, taking into consideration the anatomy and acoustics underlying vocal production. In this way, we can determine not only what information is available to receivers, but also the possible costs and benefits of producing or responding to a given signal, and thereby gaining important insights into the evolution of acoustic communication.

In my thesis, I examined the individuality and function of mother-offspring contact calls and investigated, using a sound production ("source-filter theory") perspective, the sources of acoustic variation in the sexually-selected calls of fallow deer (*Dama dama*). Fallow deer is a gregarious, size-dimorphic and polygynous deer species in which acoustic signals are produced by individuals of both sexes and at different ages. Young fallow deer (fawns) and adult females (does) produce contact calls in order to find each other when separated. Socially mature males (bucks) show a highly developed vocal display during the breeding season; producing a low-pitched, stereotyped and repetitive call, known as groan. Similarly to humans, fallow deer possess a descended larynx that males lower during vocalisation. Groans are directed towards both males and females and this suggests that they have a role in both intrasexual and intersexual contexts. Fallow deer is therefore an ideal species to address questions concerning the possible selective pressures that may have lead to the evolution of naturally-selected and sexually-selected calls in mammals.

I analysed individuality in mother-offspring contact calls and performed playback experiments to test for vocal recognition. I found that females produced individually distinctive calls and these were used by fawns for maternal recognition. By contrast, the contact calls emitted by fawns did not significantly vary among subjects and they were not individually distinguished by their mothers. Thus, in an ungulate hider species, the vocal identification process is unidirectional. In followers, both mother and offspring are able to acoustically recognise each other. I then argue that the different anti-predator strategies of newborn mammals may have shaped the modalities of parent-offspring vocal recognition.

During the breeding season, I performed simultaneous audio and video recordings of vocalising males, examining the anatomical and acoustical changes occurring within a call. I also investigated acoustic variation in groans in relation to individuality, fitness-related traits and time of the breeding season. During each vocalisation, males increased their vocal tract length on average 52% by lowering the larynx. This elongation resulted in lowered formant frequencies. The minimum formant frequencies reached when the larynx was pulled down to the maximum extent, encoded information about individual identity and body size of the caller and remain relatively stable during the breeding season. These results support the “size exaggeration” hypothesis proposed to explain the evolution of the descended larynx in cervids and other mammals, including humans. Higher-ranking males produced groans with lower minimum fundamental frequency (the lowest rate of vocal folds vibration), which may therefore carry information on the competitive abilities of the males. The changes in minimum fundamental frequency observed over the course of the breeding season may result from variation in the intensity of male-male competition and availability of mating opportunities, and the associated changes in calling rate. Decreases in the duration of groans during the breeding season are likely to indicate the declining body condition that males undergo at this time. These findings show that the acoustic components of sexually selected fallow deer groans have the potential to provide information on the identity and the overall quality of the caller. This information is likely to be used by males for mutual assessment during agonistic interactions and for mate choice by females.

ZUSAMMENFASSUNG

Akustische Kommunikation bei Säugetieren hat sich als Konsequenz natürlicher und sexueller Selektion entwickelt. Der Evolution von akustischen Signalen wird jedoch durch die Struktur des Stimmapparats und durch den Mechanismus der Lautproduktion Grenzen gesetzt. Es ist daher wichtig, die Quellen akustischer Variation zu identifizieren und dabei die der Lautproduktion zugrundeliegende Anatomie und Akustik zu berücksichtigen. Auf diese Weise lässt sich feststellen, welche Information für den Empfänger verfügbar ist, und es ist möglich, die Kosten und Nutzen von Produktion und Reaktion auf ein bestimmtes Signal abzuschätzen. So können wichtige Einsichten in die Evolution akustischer Kommunikation gewonnen werden.

In meiner Doktorarbeit untersuchte ich die Individualität und Funktion von Kontaktrufen von Müttern und deren Jungtieren, sowie die Quellen akustischer Variation in sexuell selektierten Rufen von Damhirschen (*Dama dama*) aus der Perspektive der Lautproduktion (‚source-filter theory'). Der Damhirsch ist eine herdenlebende, polygyne Hirschart mit ausgeprägtem Geschlechtsdimorphismus. Individuen beider Geschlechter und verschiedenen Alters produzieren spezifische akustische Signale. Adulte Weibchen (Kühe) und ihre Jungtiere (Kitze) geben Kontaktrufe, um sich nach zwischenzeitlicher Trennung wiederzufinden. Ab einem Alter von etwa vier Jahren zeigen Männchen (Böcke) während der Paarungszeit komplexe akustische Darbietungen bestehend aus stereotypen niedrig-frequenten Rufen, das sogenannte ‚Röhren'. Wie Menschen besitzen auch Damhirsche einen abgesenkten Kehlkopf, welchen die Böcke beim Rufen noch weiter absenken. Das Röhren ist sowohl an andere Männchen wie auch an Weibchen gerichtet, was vermuten lässt, dass es sowohl bei der intra- wie auch bei der intersexuellen Kommunikation eine Rolle spielt. Der Damhirsch ist daher eine ideale Art, um Fragen zur Evolution natürlich und sexuell selektierer Rufe bei Säugetieren zu untersuchen.

Ich analysierte Kontaktrufe von Müttern und Kitzen auf individuelle Unterschiede und führte Playbackexperimente durch um zu testen, ob diese Rufe individuell erkannt werden. Ich stellte fest, dass adulte Weibchen individuell verschiedene Rufe produzierten, und dass Kitze ihre Mütter anhand dieser Rufe erkannten. Dagegen unterschieden sich die Rufe der Kitze nicht signifikant zwischen Individuen, und Mütter erkannten die Rufe ihres Kitzes nicht. Dies bedeutet, dass die akustische Identifikation bei dieser Nesthocker-Art einseitig

verläuft. Bei Huftieren mit Nestflüchtern dagegen wurde festgestellt, dass sich Mütter und Jungtiere gegenseitig akustisch erkennen können. Ich argumentiere daher, dass unterschiedliche Strategien der Räubervermeidung von neugeborenen Säugetieren die akustische Erkennung zwischen Eltern und Jungtieren beeinflusst haben könnte.

Desweiteren führte ich während der Paarungszeit simultane Audio- und Videoaufnahmen von röhrenden Männchen durch, um anatomische und akustische Veränderungen während eines Rufes zu untersuchen. Zudem analysierte ich akustische Variationen in den Rufen im Hinblick auf individuelle und fitnessbezogene Unterschiede, sowie auf Änderungen im Laufe der Paarungszeit. Bei jedem Ruf senkten die Böcke ihren Kehlkopf und verlängerten dadurch ihren Stimmapparat im Durchschnitt um 52%, was zu tieferen Frequenzen der Formanten führte. Die tiefsten Frequenzen wurden erreicht, wenn der Kehlkopf maximal abgesenkt wurde, und enthielten Information zur Identität und Körpergrösse des rufenden Tieres. Dieses akustische Merkmal blieb im Verlauf der Paarungszeit vergleichsweise konstant, was die Hypothese der Grössenübertreibung (‚size exaggeration hypothesis'), die als mögliche Erklärung für die Evolution des abgesenkten Kehlkopfs bei Hirschen und anderen Säugetieren vorgeschlagen wurde, unterstützt. Männchen höheren Rangs produzierten Rufe mit tieferer Grundfrequenz (entspricht der niedrigsten Vibrationsrate der Stimmbänder), welche somit wahrscheinlich Information zur Kampfkraft des Tieres beinhalten. Ich beobachtete deutliche Änderungen in der minimalen Grundfrequenz im Verlauf der Paarungszeit. Dies könnte eine Folge von Unterschieden in der Intensität des intrasexuellen Konkurrenzkampfes und der Verfügbarkeit von Paarungsgelegenheiten, sowie der damit korrelierten Änderungen der Rufrate sein. Des weiteren wurden die Rufe im Verlauf der Paarungszeit kürzer, was wahrscheinlich ein Indikator der sich gleichzeitig verschlechternden Körperverfassung der Böcke ist. Diese Erkenntnisse zeigen, dass die akustischen Komponenten des sexuell selektierten Röhrens von Damhirschen das Potential haben, Auskunft zur Identität und allgemeinen Verfassung des rufenden Tieres zu geben. Diese Information wird wahrscheinlich von Männchen bei der gegenseitigen Einschätzung während agonistischen Interaktionen genutzt, ebenso wie von Weibchen bei der Partnerwahl.

INTRODUCTION

Vocal communication using both simple and complex sounds is very common among vertebrates. In mammals, acoustic signals can have widely varying information content and thus are used in many forms of social interaction. The calls produced in order to enhance survival such as alarm calls, foraging calls, and calls involved in group coordination and parental behaviour, have been shaped by natural selection, whereas calls associated with reproduction have evolved through sexual selection. Below, I describe general features of the naturally-selected calls involved in mother-offspring interactions and of sexually-selected loud calls produced by males during the breeding season. I then provide a general background about the biology and vocal behaviour of my study species. Finally I give an outline of my thesis delineating the questions I have investigated in this research.

Vocal communication and natural selection: mother-offspring contact calls

In mammals, communication and recognition between mothers and offspring is essential for offspring development and survival as females usually provide exclusive care to their own offspring. Thus, mothers and young should provide cues to establish and maintain contact (Trillmich 1981; Holmes 1990; Hepper 1991). Although olfaction appears to be of primary importance in a variety of mammals, acoustic communication has also been shown to play a crucial role in parent-offspring recognition (Balcombe 1990; Charrier 2001; Searby & Jouventin 2003). Auditory cues are expected to be particularly important in colony or herd-living animals, such as pinnipeds and ungulates, where the probability of losing contact and of mixing-up young from different mothers is high, and other sensory modalities (e.g. olfaction and vision) are not always available (Trillmich 1996; Jouventin and Aubin 2002; Searby & Jouventin 2003). In these species, mothers use olfaction and vision to confirm offspring identity only at close range. Acoustic cues are more effective over large distances and contact calls are produced by mothers and their young to find each other when they are separated (Insley 2001; Searby & Jouventin 2003).

Contact calls have been described in several species of ungulate but the ability of mothers and offspring to identify each other by means of vocal signals has been demonstrated only in few studies (Espmark 1971; Shillito-Walser et al. 1981; Searby and Jouventin 2003). These studies, together with my results, provide some evidence that mother-offspring recognition varies in relation to different anti-predatory strategies of the offspring. Two main

strategies for avoiding predators have evolved in newborn ungulates: following and hiding (Fisher et al. 2002; Caro 2005). Follower species such as domestic sheep (*Ovis aries*; Shillito-Walser et al. 1981, Searby and Jouventin 2003) and reindeer (*Rangifer tarandus*; Espmark 1971, 1974) live in open habitats and the young follow their mother from the first day of life, relying on fleeing, and maternal and group defence to avoid predation. In these species, mothers and offspring are capable of recognising each other using individually distinctive contact calls. Hider species, such as domestic goats (*Capra hircus*; Ruiz-Miranda et al. 1993) and red deer (*Cervus elaphus*; Vaňková et al. 1997), live in habitats that provide cover, and young spend most of their time hidden in vegetation to avoid detection by predators. In these species, adult females have individually distinctive contact calls but it is not known whether the calls produced by their offspring also have individual characteristics. It is also not known if mothers and their young can recognise one other based on acoustic cues. It is therefore of particular interest to extend the study of mother-offspring vocal recognition in other ungulate hider species, such as fallow deer (*Dama dama*), to understand whether different anti-predator strategies of newborn mammals may have shaped the modalities of parent-offspring recognition. The results of such studies can also help to understand whether the asymmetries in recognition depend on the dynamics of how parents interact with offspring in early life in species other than ungulates (e.g. pinnipeds) in which maternal strategies are variable and mother-offspring recognition relies heavily on acoustic cues.

Vocal communication and sexual selection: male loud calls

Male vocal display has evolved as a result of sexual selection in many species of birds, amphibians and mammals. Several experimental and comparative studies have demonstrated that individual variation in male vocal display affects individual reproductive success. Males calling for longer, more often or producing more complex calls have advantages in both intrasexual contests and intersexual display and therefore in turn have higher reproductive success than males with less elaborate vocal display (Davies & Halliday 1978; McComb 1991; Reid et al. 2005). Whereas the calling rate is usually an indicator of the motivation and intention of the caller, the acoustic structure of sexually selected calls may contain multiple messages, including information on individual identity, phenotypic traits, competitive ability and age of the caller (Galeotti et al. 1997; Castellano et al. 1999; Kitchen et al. 2003; Reby & McComb 2003). The acoustic structure describes the temporal and spectral shape of vocalisations, and it represents the physical expression of the anatomy and mechanical structure of the vocal apparatus (Fitch & Hauser 1995, 2003; Riede et al. 2005; McElligott et

al. 2006). Thus, individual morphological features of the signaller vocal system, determine the presence of individually acoustic features in the emitted signal.

Individual variation in the acoustic structure of calls permits individual recognition, and influences male-male competition and female choice. The ability to distinguish between male conspecifics based on vocal cues can help to prevent the costs of unnecessary contests (Beecher et al. 1996). Moreover, females may learn individual vocal differences and preferentially mate with familiar males who can afford longer, and therefore higher energy, courtship displays (East & Hofer 1991; Zimmerman & Lerch 1993). Information about quality-related male characteristics (such as body size, competitive ability and age) can be also encoded in the acoustic structure of calls (Fischer et al. 2004; Blumstein & Munos 2005). In toads, frogs, and birds, for example, the fundamental frequency of calls is negatively correlated with the body size of the animal (Davies & Halliday 1978; Ryan & Brenowitz 1985; Bee et al. 1999). In these species, the acoustic features of calls are constrained by body size and since large size usually confers an advantage in fights, they honestly indicate the fighting ability of the animal and more in general his resource holding potential (RHP). In loud calling mammals, evidence for a relationship between fundamental frequency of calls and body size has been equivocal (Fitch & Reby 2001; Owren & Rendall 2001). Within a given species, body size is related to fundamental frequency across age categories and among adult females, but not among adult males (Fischer et al. 2002; Collins & Missing 2003; Reby & McComb 2003; Pfefferle & Fischer 2006).

The physiological mechanisms that humans use to produce speech sounds can be applied to most mammals, for producing their calls (for a review see Fitch & Hauser 2003). According to the source-filter theory of sound production (Fant 1960), mammal vocalisations can be seen as source signals, generated by vibration of the vocal folds of the larynx and subsequently filtered in the cavities of the vocal tract. The source signal initially determines the fundamental frequency of the vocalisation. Because the larynx is not constrained by the bones of the skull, the vocal folds may grow independently of the rest of the head or body, making the fundamental frequency a poor indicator of male body size in mammals (Fitch 1997). From the larynx, the source signals passes through the supra-laryngeal vocal tract that selectively amplifies certain frequencies (formants), which then radiate out through the mouth and nostrils into the environment. Basic linear acoustics predicts an inverse relationship between the formant frequencies, the average spacing between the formants (formant dispersion) and the vocal tract length (VTL), which is in turn related to the overall body size

of the animal (Titze 1994; Fitch 1997). Frequencies produced by the filter can vary independently from the frequencies produced by the source, so that one or both have the potential to provide receivers with important information on the identity, body size or other indirect measures of male fitness (e.g. dominance rank and mating success) of the caller.

One of the most fundamental questions regarding the development of any communication signal is the extent to which its structure is relatively fixed over time. Short-term vocal changes may reveal the presence of dynamic acoustic features that may convey information about aspects of the current condition of the caller (Scheuber et al. 2003; Loyau et al. 2005). It is important to study the variation of individual vocal behaviour or call characteristics over short time-scales in order to identify stable and dynamic acoustic characteristics. Short-term vocal changes may in fact have important implications for vocal individual recognition and male assessment. Moreover, stable and dynamic vocal characteristics may reflect different selection pressures acting on male vocal display and may be used differently by females and rival males.

Gregarious and polygynous deer species provide an interesting model for investigating the role of the acoustic structure of sexually selected calls in mammal communication, and testing hypotheses concerning the evolution of honest vocal signalling in male-male competition and female choice. Males of these species are silent for most of the year, and then have an extremely intense period of vocal activity associated with the breeding season (Clutton-Brock & Albon 1979; McElligott et al. 1999). They produce loud calls that are directed both towards males during aggressive contests and females during chasing or herding behaviour, suggesting a role of these calls in both intra- and inter-sexual contexts. Recent work has also revealed that males of some species have a descended larynx, which is a characteristic of the vocal apparatus that is shared with humans and few other mammals (Fitch & Reby 2001). During vocalisation, males pull down the larynx towards the sternum and this allows them to increase their vocal tract length. In red deer, this leads to a decrease in the minimum formant frequencies, which provide an honest indication of body size (Reby & McComb 2003). This information is perceived by both females and males and used by the latter in competitive interactions (Reby et al. 2005; Charlton et al. *in press*). Females prefer males producing lower formants as well as higher calling rates (McComb 1991; Charlton et al. 2007).

The study of the evolutionary mechanism that has led closely-related species of deer to have diverse vocal behaviour (in terms of calling rate, vocalisation posture, and acoustic structure of calls) is important to understand the convergence or divergence of vocal signalling in other species. A better understanding of these mechanisms can lead to rich insights into the evolution of vocal communication. In my thesis I examine the factors underlying acoustic variability in the sexually selected calls produced by fallow deer males in relation to individuality, male quality, and short-term changes. I discuss the possible different selective forces that have shaped the evolution of male vocal display in a species in which one of the main modes of communication during the breeding season is vocalisation.

Study species

The fallow deer is a medium-sized, highly polygynous deer species originating from south-eastern Europe (Chapman & Chapman 1975). Like in other group-living animals, communication based on auditory cues is likely to play an important role in social interactions. In fallow deer, acoustic signals are used by individuals of different ages and sexes in different social contexts. When they are fawns, both males and females emit naturally-selected contact calls. Young fallow deer lie concealed and silent in vegetation, and separated from the mother for their first weeks of life. During this time, mothers return to their fawns intermittently to feed them. When a mother approaches the approximate location of the fawn, she starts to vocalise until the reunion occurs. Two or three weeks after birth, fawns follow their mothers in large mixed herds of females and other fawns, and start vocalising more frequently in order to find their mothers (Gilbert 1968; Braza and San Josè 1988).

From weaning until they are old enough to compete with rivals, adult males remain largely silent (McElligott et al. 1999). They become socially mature at the age of four when they start to participate in activities associated with reproduction (Moore et al. 1995; McElligott et al. 1998). The breeding season for fallow deer in the northern hemisphere lasts from September to early November. Most matings take place during the last two weeks of October (McElligott & Hayden 2000). Male mating strategies are variable, ranging from territorial to non-territorial (Clutton-Brock et al. 1988; Apollonio et al. 1992; Moore et al. 1995; Thirgood et al. 1999). Mating success is highly skewed (McElligott & Hayden 2000) and strongly related to dominance rank. Body size is important to determine the rank and therefore the mating success of the males (McElligott et al. 2001). Fallow deer is a highly size

dimorphic deer species with mature males weighing approximately two times more the females (McElligott et al. 2001). This strong size dimorphism is already evident at birth due to male-biased maternal investment (Birgersson & Ekvall 1997; Birgersson 1998; Birgersson et al. 1998). Because of a large reduction in feeding and greatly increased investment of time and energy in vocalising and fighting in order to gain matings, males lose approximately 26% of their body weight during the breeding season (McElligott et al. 2003; Apollonio & Di Vittorio 2004).

Males start to vocalise approximately 3 weeks before the first mating occurs and continue until the end of the rut. During this time, they produce low-pitched calls known as groans. Long-term investment in vocalisation is important to obtain a high reproductive success (McElligott et al. 1999) and this suggests that familiarity may play an important role in female mate choice in this species. Males vocalise at rates that are among the highest recorded for any terrestrial mammal (McElligott & Hayden 1999). Short-term variation of the groaning rate is thought to be a threat signal mainly directed to competitors (McElligott & Hayden 1999). Males possess a descended and mobile larynx (Fitch & Reby 2001; McElligott et al. 2006) and during vocalisation, they lower the larynx, but never to its maximum possible extent (the sternum). Towards the end of the breeding season, groaning rates are reduced and groans sound hoarse, possibly because of exhaustion. These vocal changes may therefore be related to the current condition of the males and represent valuable information for other males assessing the current competitive abilities of their rivals.

Outline of the thesis

In my thesis I address questions regarding the potential for information transfer as a result of the acoustic structure of calls used during reproduction, and during mother-offspring interactions in fallow deer. I used the source filter-theory of voice production as a basis for the analysis of the calls and interpretation of the results. In **Chapter 1**, I first give an overview of the different types of calls produced by deer according to different behavioural contexts in which they are emitted. Then, I describe the methodological approach we applied to investigate the functional significance of naturally- and sexually-selected calls in fallow deer, highlighting the difficulties we encountered and possible solutions to adopt to solve the problems. In **Chapter 2**, I investigate mother-offspring acoustic recognition by quantifying intra/inter-individual variation in the calls of both mothers and young fallow deer and testing for the existence of vocal recognition using playback experiments. I show that in fallow deer,

mother-offspring recognition based on acoustic cues is a unidirectional process as only fawns can acoustically recognise their mother and not vice versa. I interpret the main result in the context of the hider anti-predator strategy adopted by newborns in this species. In **Chapter 3**, I turn to the acoustic structure of sexually selected calls produced by males and show that the lowering of the larynx during vocalisation results in lowered formant frequencies. This result not only demonstrates that the frequency bands of groans are formant frequencies, but also gives further weight to the "size exaggeration" hypothesis, proposed to explain the evolution of descended larynx in mammals, including humans. In **Chapter 4**, I investigate if enough acoustic variation exists to potentially allow individual recognition based on groans, and the relative importance of different call features for achieving individuality. I suggest that in contrast to what has been shown in other mammals, such as humans and other primates, source and filter-related features contribute equally to the vocal identity, possibly because of the different selection pressures acting on the acoustic structure of groans. In **Chapter 5**, I examine if the acoustic structure of groans can potentially provide accurate information on the quality of the caller by investigating the relationships between the frequency components of groans and male fitness-related traits such as body size, dominance rank, and mating success. I show that formant frequencies can honestly signal male body size, whereas fundamental frequency is related to aspects of competitive abilities. I suggest that this information is likely to be used by males for mutual assessment during agonistic interactions and as a cue for mate choice by females. In **Chapter 6**, I investigate if changes in the acoustic structure of groans and groaning rate occur over the course of the rut. The results reveal the presence of both stable and dynamic acoustic features in fallow deer groans suggesting that groaning represents a multicomponent signal.

This study provides a solid base for the understanding of the complexities of vocal signalling in what is probably the most vocal of any deer species. It gives novel insight into parent-offspring recognition in ungulates and contributes to the understanding of how these mother-young vocal discrimination systems may have evolved. Moreover, it sheds light on the possible different selective forces that have shaped the evolution of sexually-selected mammalian loud calls. Finally, my research represents a contribution to the comparative study of vocal communication in mammals at the basis of the understanding of the origin and evolution of human speech.

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CHAPTER 1

Acoustic signaling in cervids: a methodological approach for measuring vocal communication in fallow deer

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Acoustic signaling in cervids: a methodological approach for measuring vocal communication in fallow deer

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ABSTRACT

In deer, like in other group-living mammals, communication plays a crucial role in social interactions. Acoustic signals are particularly widespread and used by individuals of all ages and sexes in different social contexts. Over the past 20 years the interest in deer vocal communication has increased with the development of new techniques in sound recording and acoustic analysis. In this paper, we present two studies on vocal communication in fallow deer and discuss the methodological approach we applied to investigate and test the information content of calls used during reproduction, and mother-offspring interactions.

INTRODUCTION

Communicative behavior occurs in any form of social interaction and implies an exchange of information between at least two individuals; a signaler and a receiver. The acquisition and the use of information helps animals to anticipate and respond appropriately to events, and therefore to increase their survival (Owings et al. 1997). For example, through communication, social dominance hierarchies are established without dangerous fights occurring, members of groups warn each other about potential danger and recognition occurs between mothers and their young (Halpin 1991; Huntingford et al. 2000). In communication, information is made available by signals that vary in relation to the type of information delivered and to the surrounding environment. Deer are group-living animals characterized by a definite social structure in which communication plays a central role in affecting and regulating all social interactions between the members of the group. Deer rely on visual, olfactory and auditory cues to communicate and they may also use different kinds of signals in combination to reinforce a particular response. Auditory exchange is less hindered by obstacles than vision, and vision is also of limited value in dim light or at night. Moreover, although sounds lack the durability of chemical signals, they can be transmitted both over short and long distances (Marler 1977). Vocal signals constitute a very efficient system of communication and are widely used by deer in a variety of circumstances.

Vocal communication in deer

Several types of vocalizations have been described in all studied species and generally classified according to the context of utterance (de Vos et al. 1967; Clutton-Brock & Albon 1979; Richardson et al. 1983; Bowyer & Kitchen 1987; Oli & Jacobson 1995; Vaňková & Málek 1997; Reby et al. 1999).

1. Vocalizations in situation of danger

1.1. Alarm calls

Alarm calls are given by mature and yearling deer of both sexes when danger is detected. There is great species-specific variety in the structure and in the way in which these calls are produced. They range from dog-like barks emitted through the mouth to alert snorts emitted through the nose. In solitary deer the main function of these call is to inform the predator that it has been detected and therefore to discourage it from attacking the prey (Hirth & McCullough 1977; Yahner 1980; Reby et al. 1999).

1.2. Distress calls

Distress calls are emitted during times of acute distress, such as during an attack by a predator or when an animal is critically injured. The motivation is specifically the fear of being captured and/or dying. The call is usually long, uncontrolled, very loud and given with the mouth open. Deer of both sexes and all ages can produce these high-pitched vocalizations that are relatively similar in all species (Espmark 1974; Richardson et al. 1983; Atkeson et al. 1988; Vaňková & Málek 1997). Distress calls are probably directed non-specifically and induce strong reactions in all conspecifics that become instantaneously and acutely alerted when they hear these sounds.

2. Vocalizations during reproduction

2.1. Reproductive calls

In monogamous or slightly polygynous deer, vocalizations during the reproductive period are mainly given by males during agonistic encounters. These agonistic calls vary according to the degree of arousal of the animal or to the intensity of the interaction (Atkeson et al. 1988). Evidence for calls given by males during courtship is scarce and in most cases related to the production of soft and short vocalizations (Atkeson et al. 1988; Geist 1990; Danilkin & Hewison 1996).

In gregarious and polygynous deer, males compete intensely for females and show a highly developed vocal display during the breeding season. In these species, male vocalizations cannot be usually attributed to specific actions such as aggression or sexual activities, but instead reflect the motivational state of the animal. The acoustic structure of these calls varies across different species and subspecies (Reby & McComb 2003*a*), but they are generally loud and repetitive and therefore suitable to emphasize the presence of the individual and to advertise its position at considerable distance.

3. Vocalizations in mother-offspring interactions

3.1. Contact calls

Communication between mothers and offspring represents a highly individualized process due to the strong bond between the two partners involved. Mothers and their offspring emit contact calls in order to find each other when they are separated. Vocalizations produced by offspring are typically high-pitched and characterized by a harmonic structure, while mothers produce sounds of moderate pitch and low tonality (Atkeson et al. 1988). Contact calls have

been described in several species of deer and in some the ability of mothers and offspring to identify each other by means of vocal signals has been demonstrated (Espmark 1971, 1974; Vaňková et al. 1997).

3.2. Nursing whine

Young deer produce a call known as a nursing whine while they suckle their mothers. This call is generally brief, of low intensity with varying tonality and may serve to identify the offspring or reinforce the maternal bond (Richardson et al. 1983; Atkeson et al. 1988; Bowyer & Kitchen 1987).

Why studying vocal communication in deer?

During the last 20 years, the study of animal vocal communication has made a lot of progress because of technical advances in recording equipment and acoustic analysis. Compared to visual and olfactory signals, acoustic signals are easier to quantify and to test with playback experiments in order to verify their effective role in communication. However, despite the increasing number of studies on vocal communication in ungulates, not much work has been carried out on wild deer. Moreover, most of this work is descriptive and the functions of the calls are simply deduced from the context of occurrence (Bowyer & Kitchen 1987). Only recently, researchers started to examine communication in detail by investigating the type of information conveyed during vocalization, and if this information is really used by the animals to communicate (Reby & McComb 2003*b*, Reby et al. 2005).

The purpose of this paper is to introduce the reader to the study of vocal communication in deer and specifically to a methodological approach that can be applied to address questions about the information contained in vocalizations. We describe two case studies in which we investigated the structure and possible function of calls involved in reproduction, and mother-offspring communication in fallow deer (*Dama dama*), by using detailed acoustic analyses and playback experiments. For each study we first give a general introduction about the vocal behavior of fallow deer in the specific communicative context and we mention the aims of the research. Then, based on our work, we suggest methods that can be used in these types of studies.

FIRST CASE STUDY: INFORMATION CONTENT OF FALLOW DEER

The fallow deer is a medium-sized, highly polygynous and size dimorphic deer species (Chapman & Chapman 1975). Fallow bucks only vocalize during the breeding season from the end of September to early November. In this time, socially mature males (≥4 years old) invest time and energy in vocalizing and fighting in order to gain matings and they can lose approximately 26% of their body weight (McElligott et al. 2003). Fallow bucks produce a loud, stereotyped and repetitive call known as groan, and they can also groan at rates of more than 3000 times per hour (McElligott & Hayden 1999). Groans are individually distinctive (Reby et al. 1998) and as is the case with red deer, conspecifics are also likely to be able to recognize males based on the sound of their calls (Reby et al. 2001). Groans are directed both towards females during approaching, chasing or herding behavior, and males during agonistic contests. Previous research suggests that the long-term investment in vocal signalling represents the overall quality of the signaller and is directed at females (McElligott et al. 1999). By contrast, variation in short-term vocalization rates (groans per minute) are thought to transmit information mainly to other males (Komers et al. 1997; McElligott & Hayden 1999, 2001). This suggests that call rate could be used as an assessment cue in competitive interactions.

Although calling rate may reflect the condition of the displaying animal, additional information about individual quality may be encoded in the acoustic structure of calls. We investigated the role of the phonic structure of fallow buck groans as a potential source of information about male quality, by examining the relationships between signal components and characteristics of signaler such as body size and mating success. In species in which male vocal display has evolved as a result of sexual selection, we expect vocal signals to convey information about caller attributes that play an important role in male-male competition and mate attraction. Body size is certainly of crucial importance in all species in which males engage in fights in order to establish social dominance. In fallow deer as well as in many other sexually dimorphic ungulates, larger males have advantages over other males when competing for matings (McElligott et al. 2001). In addition to body size, some other factors such as physical condition may also affect vocalizations and explain some of the variation in male mating success and dominance rank. Body size and mating success were measured according to McElligott et al. 2001.

METHODOLOGY

1. Study place & population

Research on bioacoustics usually requires the possibility to record, observe and identify the animals at relatively close distances. Since deer are generally elusive animals, populations living in farms and parks have been mainly used to investigate their vocal behavior (Yahner 1980; Richardson et al. 1983; Vaňková & Málek 1997; Reby et al. 2001). However, farms do not represent suitable study locations to conduct research that requires data on males. This is because farms usually keep only one or a few males and the dominant male is generally the only one that vocalizes. Whether a park or a free-ranging population is chosen to carry out the research, it is important to verify that the accessibility to the animals satisfies the requirements of the study. In our case, it was important to be able to identify the males, to collect data about their physical characteristics and their behaviors and to get good recordings on a number of different individuals. Field work was carried out on a herd of European fallow deer in Phoenix Park, Dublin, Ireland (53°22'N, 6°21'W). Approximately 600 deer live in semi-natural conditions in this large city park (709 ha) where natural forage is abundant (Hayden et al. 1992). Most of the deer are marked with ear tags and therefore individually recognizable. The animals are habituated to people and therefore we could carry out sound recordings and behavioral observations at distances of between 10 and 50 meters. Moreover, because most of the area consists of open pasture, it was possible to observe the deer and catch them in order to take body measurements.

2. Sound recording

2.1. Equipment and recording

We recorded the calls using a digital audio tape (DAT) recorder (Sony TCD-D100) and a directional microphone (Sennheiser MKH 70). This is the equipment that is usually used to record vocalizations in deer. Before starting any recording, we set the sample frequency at 48 KHz and the recording level in manual mode on the DAT. We also checked that the time and the date were correct because these data are impressed on the tape automatically during each registration. At the beginning of different recordings, we registered the identity of the animal and the context of utterance on the tape and subsequently on a notebook. This is a really important step to remember in order to be able to identify and use all the recordings afterwards.

Recordings of good quality are those characterized by a high signal to noise ratio. To maximize this ratio, we got close to the animals and set the level of recording as high as possible, without overloading the signal. When the signal is overloaded, its loudness is too high for the sensitivity of the microphone and this produces distortions in the signal. After each recording session, we listened to the tape to determine if a change of the recording level for the next session was needed.

2.2. Factors influencing the quality of the recordings

Background noise is very common problem in bioacoustics and it can affect the quality of the recordings in a serious way. For example, despite being relatively close to the vocalizing animal, our recordings were not always of good quality. Especially during windy days, Phoenix Park was characterized by a high level of background noise, principally due to car traffic. Because the low frequencies of this background noise overlapped with the low frequencies of the fallow deer groans, in some cases the frequency patterns of the calls were impossible to analyze. We could not use any filter (see the second case study) to improve the quality of the recordings because in our case filtering out the frequencies of the noise would also remove and alter some of the frequencies of the signal. The use of a windscreen cover for the microphone helped us to minimize the amount of background noise on the recordings. The position of the vocalizing animal to the microphone may also affect the quality of the signal. When the groaning male was facing the microphone, the signal was complete. However, in other conditions such as when he was moving away, some of the frequencies did not appear in the signal.

3. Signal acquisition and acoustic analysis

3.1. Signal acquisition

The first step in digitizing a sound file is to connect the audio source (e.g. DAT recorder) to the computer. The connection is usually done by plugging an audio cable from the line output on the tape recorder to one of the input lines on the computer or sound card. After we connected the DAT to the computer, we used Avisoft SAS Lab-Pro 4.32 to import the sounds. Groans were digitized at a sample frequency of 22 KHz and saved in WAV format, at 16 bits amplitude resolution.

3.2. Exploration of the signal

Before starting with the acoustic analysis, recordings should be first explored in order to get an initial impression of the temporal and spectral structure of the sounds. We first down sampled the sound files to 16 KHz for a better frequency resolution and then we generated narrow-band spectrograms using Avisoft SAS Lab-Pro 4.32 (The manual for the program is available at http://www.avisoft-saslab.com/SASLabPro.pdf). For normal applications, spectrogram parameters should be set as follows: Hamming window, FFT size from 128 (for good temporal resolution) to 512 (for good frequency resolution), overlap of 50%, and frame size of 100%.

3.3. Selection of calls and acoustic parameters

Only calls with a low level of background noise were included in the acoustic analysis. Moreover, for each male, we selected groans from different bouts so that the data can be considered independent. The types of acoustic parameters selected for the analysis depend to a large extent on the questions to be answered. We measured fundamental frequency- and formant-related acoustic features since these parameters are likely to vary accordingly to the length of vocal apparatus (see “source-filter” theory of sound production, Fant 1960) and therefore in turn to the overall body size of the animal (Fitch & Hauser 2002).

3.4. Acoustic analysis

Groans are short and low-pitched vocalizations, characterized by a train-pulse structure (figure 1). In these types of calls, fundamental frequency (F0) is defined as the inverse of the pulse duration and this can be measured as the distance between the peaks of two subsequent pulses. Distances between pulses were measured automatically from the envelope (amplitude vs. time) of the signal by applying the “Pulse Train Analysis” function in Avisoft SAS Lab-Pro 4.32. We calculated the values of the F0 along the call as the inverse of the pulse durations. The following F0-related parameters were included in the analysis: mean F0, minimum F0, and maximum F0. We also calculated the variability of F0 along the call (Jitter) as the ratio between the standard deviation to mean of F0 per call (Riede & Zuberbüler 2003). Mean F0 can be also calculated as the pulse rate of the call. This is given by the ratio between the length of the call and the number of pulses that call contains (Sanvito & Galimberti 2000).

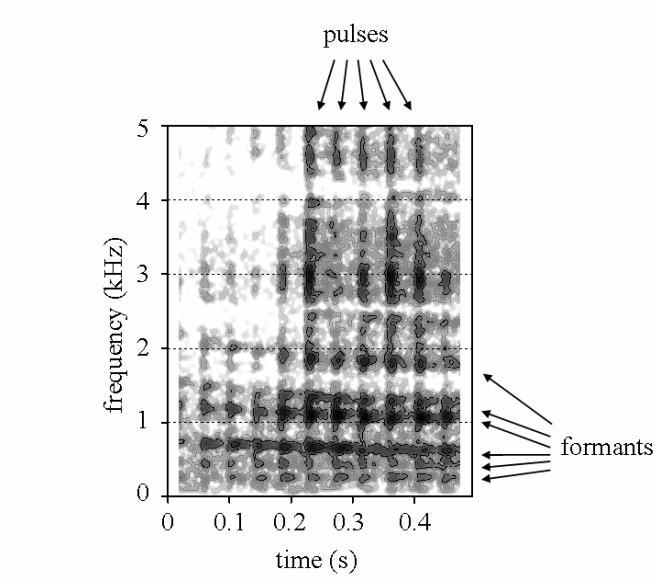

Figure 1. Spectogram of a fallow deer male groan. Pulses and formants are indicated.

From the spectrogram of figure 1, six formants (vocal tract resonances) are clearly visible. From the third formant upwards, frequencies decrease along the call until they reach a minimum value at the plateau. This change in formant frequencies represents the elongation of the vocal tract of the fallow buck during vocalization, as a result of the movement of the larynx down towards the sternum (Fitch & Reby 2001, Birrer et al. in preparation). Formant frequencies of groans were estimated by using Linear Predictive Coding (LPC) analysis in Praat 4.3 DSP package (available from Paul Boersma and David Weenink, www.praat.com). The LPC model is based on a mathematical algorithm from which the vocal tract resonances underlying the overall sound spectral energy pattern can be derived (see Markel & Gray 1976 for mathematical details). This method has only been used in speech research for a long time but it represents a valid and powerful alternative technique to the traditional frequency spectrum analysis for measuring formants in animal sounds (Owren & Bernacki 1988). We measured the mean and the minimum frequency values of the first six formants in each groan. The minimum formant frequencies were calculated by averaging the values over the last part of the call (plateau). To create a new LPC from every selected sound we use “To formants (Burg)” command (for details about formant analysis see http://www.fon.hum.uva.nl/praat/manual/Intro_5__Formant_analysis.html). LPC measurements were transferred to Excel and plotted against time and frequency. A superimposition of these plots over the spectrograms of each groan allows the researcher to eliminate spurious values and to verify the output of the analysis. We also estimated minimum formant dispersion (minimum spacing of the formants) from the minimum formant frequencies of each groan according to Reby & McComb (2003b).

SECOND CASE STUDY: THE ROLE OF AUDITORY CUES IN MOTHER-OFFSPRING RECOGNITION IN FALLOW DEER

Fallow deer is a typical hider species (Chapman & Chapman 1975), meaning that for the first two to three weeks of life, fawns spend most of the time lying concealed and silent in vegetation (Langbein & Putman, 1992). Hiding is an anti-predator strategy that evolved in ungulates, which live in habitats providing cover (Lent 1974). Mothers remain separated from their hidden fawns and return to them several times per day for suckling. Because fawns choose the hiding places, mothers do not know the exact locations of their fawns. When a mother is ready to nurse, she moves towards the location where the last contact with her fawn occurred and then she starts to vocalize. The fawn gets up, sometimes calls back, and comes to her. The mother smells the fawn for a final identity check before allowing it to suckle. After the fawn has fed, it goes back to hide. As fawns grow, they progressively join the herd (Chapman & Chapman 1975). Because of the visual confusion, vocalizations are supposed to play a fundamental role in keeping mothers and fawns in contact within the group.

We investigated the role of vocalizations in the recognition process between fallow deer mothers and fawns. We first conducted a detailed acoustic analysis of mother and fawn calls in order to identify the parameters that can potentially code for individuality and therefore allow recognition. We then tested if mothers and fawns are able to recognize each other based on vocalizations by performing playback experiments.

1. Study place & population

We conducted our study on a farm population of fallow deer. The animals were tagged and individually identifiable. They were also habituated to humans and therefore it was possible to observe and record them at relatively close distance (15-50 meters) without affecting their behavior. Compared to parks or wild reserves, deer on farms often live in small herds within enclosures. This allows the researcher to observe all of them simultaneously and to follow single animals when the group is moving. In our case, the herd consisted of 20 females with their offspring and 2 adult males. The size of the enclosure (4 ha) and the availability of natural cover (such as high grass and tree branches) allowed fawns to hide during the first weeks of life. From a raised observation location on one of the sides of the enclosure, we could easily observe the animals and we were able to detect where the fawns were hidden.

2. Sound recording

2.1 Equipment and recording

The equipment and the settings used during recording were the same as those described in the first case study.

2.2. Factors influencing the quality of the recordings

In this study, background noise did not represent a problem since we were able to apply filters to our recordings (see below). However, when an adult female started to vocalize, many other adult females also became vocal. This occurred every few hours when many fawns were simultaneously nursed. Recordings taken at these times showed high levels of overlap between the calls of different individuals, and this made the identification of the recorded animals difficult. In order to minimize this problem, recording of isolated mothers or fawns should be attempted if possible. In general, to collect sufficient recordings of all individuals, it is necessary to spend long hours in the field.

3 Signal acquisition and acoustic analysis

3.1. Signal acquisition

The process of signal acquisition was the same as the one described in the first case study.

3.2. Exploration of the signal

In order to explore the signal, we generated spectrograms from each call using Praat 4.3 DSP. We set the spectrogram parameters as follows: window length = 0.01 s, time step = 1000, frequency step = 250, frequency resolution = 20 Hz, Gaussian window shape, dynamic range = 35 dB (for details about how to make spectrograms in Praat see http://www.fon.hum.uva.nl/praat/manual/Intro_3_2__Configuring_the_spectrogram.html).

3.3. Selection of calls and parameters

In order to select calls of good quality, we first filtered our sound files using the “Filter (stop Hann band)” command in Praat. This method allows the elimination of a frequency band from the spectrogram. Setting the lower edge of the stop band to the value zero, this filter acts as a high-pass filter. The upper edge has then to be set at a value that allows excluding as much background noise as possible, but keeping the lowest frequencies of the calls intact. We could use these filters because the maximum frequency of the background noise (about 100-130 Hz) was below the minimum frequency of the calls, and therefore the signal and the noise did not

overlap. We then selected calls where the profile of the fundamental frequency (F0) was clear along the entire vocalization. For the acoustic analysis, we measured frequency-related parameters (extracted from the F0 profile and from the formant pattern), temporal parameters (such as call duration), and parameters related to the distribution of energy along the call (extracted from the intensity contour and from the average power spectrum). These parameters have been already used to investigate the potential for individuality contained in vocalizations of ungulates (Searby & Jouventin 2003).

3.4. Acoustic analysis

Contact calls of fawns are tonal with a clear harmonic structure (figure 2*a*). The vocalizations of mothers are of three types: calls composed of a first noisy part and a second part with harmonic structure (figure 2*b*), tonal calls (similar to fawn vocalizations), or completely noisy calls.

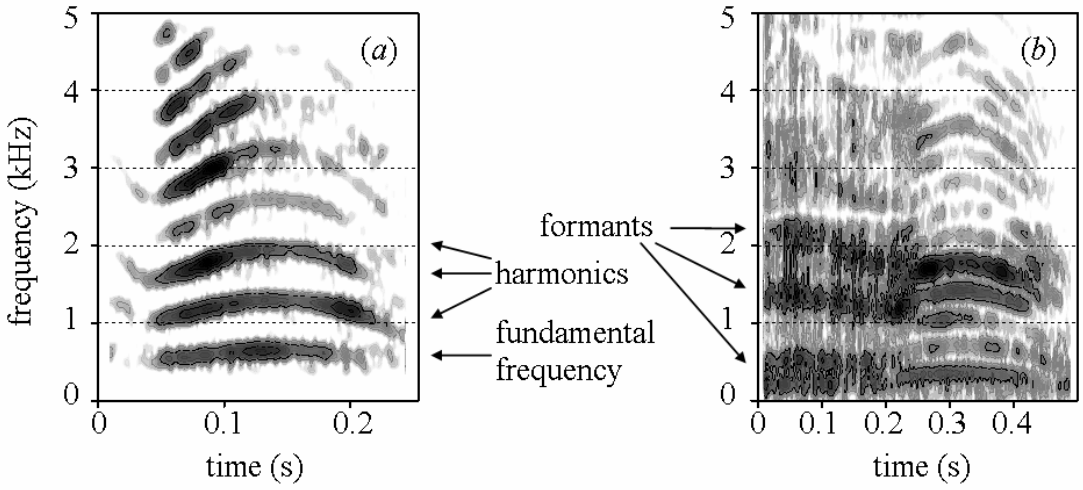

Figure 2. Spectrograms of typical contact calls of fawns with well-defined harmonics (*a*) and of a mother (*b*) with a noisy part first and a second part with harmonic structure.

We used Praat 4.3 DSP to analyze both mother and fawn calls (for details of the analysis, see the manual of the program at http://www.fon.hum.uva.nl/praat/manual/Intro.html). The F0 profile of each call was produced using a pitch analysis ("To pitch (ac)" command). This method is based on an autocorrelation algorithm associated with filtration of the signal as described in Boersma (1993). We used these frequency contours to measure F0 mean, F0 initial, F0 final, F0

minimum, F0 maximum and temporal parameters such as the time of F0 minimum, time of F0 maximum, and the duration of the harmonic part of the call.

In order to characterize the formant pattern of mother calls we used Linear Predictive Coding analysis (see the first case study). We extracted the mean frequency of the first three formants and measured the duration of the formant part for each call.

From the Intensity contour ("To intensity" command) of the call we measured the maximum intensity, the mean intensity and the time when highest amplitude peak occurs. We also displayed the average power spectra ("To spectrum" command) of the calls in order to analyze the distribution of the energy among frequencies. From these spectra we identified the first three bands with energy peak and measured their relative frequencies.

4. Playback experiments

4.1. Plan and execution

We used playback experiments to verify the potential of the acoustic structure of calls to code for individuality. The first step was to plan the experimental design and to carry out a pilot study to verify its feasibility. Preliminary experiments are useful to decide which set-up should be used (distance between the loudspeaker and the animals, volume of the playback sequence, position of the video camera).

We conducted playback experiments on 12 mother-fawn pairs. Before each playback, we waited until the mother-fawn pair had lost visual contact with each other, and approximately two hours had passed since the last suckling occurred. In this way, all individuals were motivated to respond. Calls were played back directly from a laptop connected to a battery powered loudspeaker (Sound Projections Sound Machine SM-2, 200W, frequency response 65 Hz- 16 kHz), placed 15-50 m away from the tested animal. During playbacks, the loudspeaker and researchers were concealed from view. Based on the observed natural occurrence of the reunion processes between mothers and their fawns, we decided to perform no more than two playback experiments per day. The animals were tested twice during the entire study period with two weeks between the two sessions to avoid the habituation of the animals. Habituation is defined as the temporary disappearance of an innate response when it is elicited many times in succession (McGregor et al. 1992) and it is a problem to keep in mind during the design of the experiments. Playback sequences were

separated by two minutes of silence. During this time the animals returned to their previous activity and we could evaluate the reactions of the tested individual to different sequences. Each sequence was composed of calls from the same animal, and a minimum of half of the calls were different from each other in order to prevent pseudoreplication. The problem of pseudoreplication arises when the data used in a statistical test are not appropriate for the hypothesis being tested (McGregor 2000). In playback experiments, this happens when treatments are not replicated or replicates are not independent (Kroodsma et al. 2001). In general, pseudoreplication is a common problem in field experiments (Hurlbert 1984; Wiley 2003) and it is important to be aware of it during the planning of the experiments. The behavior of the experimental subjects was videotaped using two Sony digital video cameras (DCR-TVR 50E) during the entire playback session. Video recording was also carried out for few minutes before and after each playback as control.

4.2. Extraction of the data from the video tapes

We classified the reactions of the tested animal in a binary way since we only needed to know whether mothers and fawns would respond or not to playback signals. This method was also applied in other studies of parental recognition (Charrier et al 2002; Searby & Jouventin 2003). The ethological scale we used to classify reactions was the following: negative reactions were defined as no behavioral changes; positive reactions were when the deer turned its head with ears orientated toward the loudspeaker, called in reply or moved toward the loudspeaker.

CONCLUSIONS

The study of vocal communication in deer should involve a combination of acoustic analysis and playback experiments. Vocalizations that are involved in sexual selection contexts are likely to contain information about characteristics of the signaler. In cases where signaler and receiver must recognize each other, calls should also contain information about individuality. Programs that are normally used in speech research should be considered for investigating the phonic structure of animal vocalizations since they allow the application of advanced techniques in acoustic analysis. Once the information content of calls has been examined using acoustic analysis, its use in communication should be tested through playback experiments. The significance of the signal can be deduced from the response elicited in the

receivers, by playing back calls with different characteristics. Finally, experiments with re-synthesized sounds may be used to test the function of all the components of the call.

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CHAPTER 2

Mother-young recognition in an ungulate hider species: a unidirectional process

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Mother-young recognition in an ungulate hider species: a unidirectional process

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ABSTRACT

Parent-offspring recognition is usually crucial for survival of young. In mammals, olfaction often only permits identification at short range, and vocalizations are important at longer distances. Following and hiding antipredator strategies found in newborn mammals may also affect parental recognition mechanisms. We investigated mother-offspring recognition in fallow deer, an ungulate hider species. We analyzed the structure of adult female and fawn contact calls to determine whether they are individually distinctive and tested for mother-offspring recognition. Only females (and not fawns) have individualized vocalizations, with the fundamental frequency as the most distinctive parameter. Playback experiments showed that fawns can distinguish the calls of their mothers from those of other females, but mothers could not discriminate their own and alien fawn calls. Thus, the vocal identification process is unidirectional. In followers, mother-offspring acoustic recognition is mutual, and therefore the different antipredator strategies of newborn mammals may have shaped the modalities of parent-offspring acoustic recognition.

INTRODUCTION

Sophisticated parent-offspring recognition is essential when parental investment is large and in colony- or herd-living animals where the potential exists for identification errors (Trivers 1972; Trillmich 1996; Jouventin and Aubin 2002). The most important component of parental care in mammals is lactation (Pond 1977; Gittleman and Thompson 1988). Females usually display selective maternal investment by restricting care to their own offspring and rejecting others, although milk stealing and/or allosuckling can also occur (Roulin 2002). The ability of mothers and offspring to recognize each other is therefore crucial to avoid misdirected maternal investment and to ensure survival of young.

Two main strategies for avoiding predators in the first weeks of life have evolved in newborn ungulates and macropods: following and hiding (Fisher et al. 2002; Caro 2005). Follower young are mobile soon after birth and therefore rely on fleeing and maternal and group defense to avoid predation. Hider young spend most of their time hidden in vegetation to avoid detection by potential predators. The follower strategy evolved in species that live in open habitats, whereas hiders live in habitats providing cover (Fisher et al. 2002). It is possible that these two widely differing strategies may also have affected the vocal recognition process of mothers and offspring because of the large differences in the way mothers and offspring of follower and hider species interact during the first weeks of life.

The recognition process between ungulate mothers and their offspring mainly involves olfaction and audition (Searby and Jouventin 2003). Olfaction supports recognition only at short range and is used by mothers for a final check of offspring identity before allowing suckling. Acoustic signals are efficient over both short and long distances, and therefore vocal communication appears to be a key factor for mother-offspring recognition (Searby and Jouventin 2003). Vocal communication is particularly important for ungulate hider species because mothers and offspring rely on calling for reunions to occur when offspring are hidden.

There is already some evidence to suggest that mother-offspring recognition varies in relation to different antipredatory strategies of the offspring. In follower species such as domestic sheep (*Ovis aries*; Shillito-Walser et al. 1981; Searby and Jouventin 2003) and reindeer (*Rangifer tarandus*; Espmark 1971, 1974), mothers and young are capable of recognizing each other using contact calls. Less is known about hider species; in domestic

goats (*Capra hircus*; Ruiz-Miranda et al. 1993) and red deer (*Cervus elaphus*; Vaňková et al. 1997), adult females have individually distinctive contact calls that can potentially allow individual discrimination. However, it is not known if adult females of hider species are able to identify their offspring using contact calls, or if offspring can recognize their mothers using contact calls.

We investigated mother-offspring acoustic recognition in fallow deer (*Dama dama*), an ungulate hider species. Young fallow deer lie concealed and silent in vegetation, and separated from other conspecifics for their first 2–3 weeks of life. Mothers spend most of the time away from the place where their fawns are hidden and return intermittently to feed them. Females usually do not know the exact locations of offspring hiding places. Therefore, a mother first approaches the approximate location and vocalizes, and then the fawn joins the mother for suckling. After the initial hiding phase, fallow deer fawns follow their mothers in large mixed herds of females and other fawns, and fawns also vocalize more frequently (Gilbert 1968; Braza and San Josè 1988).

First, we analyzed the acoustic structure of adult female and fawn contact calls to determine whether they were individually distinctive (Reby and McComb 2003; Searby and Jouventin 2003). Fallow deer fawns are sexually size dimorphic at birth, and males also grow faster than females (Birgersson and Ekvall 1997). Therefore, we also examined age and sex differences in fawn contact calls because these factors could affect the potential for recognition. Using playbacks, we then experimentally tested the capabilities of mothers and fawns to recognize each other's vocalizations.

METHODS

Study Sites and Animals

This study was conducted from June 4 to September 1, 2004, using two herds of European fallow deer on farms. These herds consisted of 21 adult females and 20 fawns located in Oberembrach (Canton Zurich, Switzerland) and 13 adult females and 6 fawns in Paspels (Canton Graubünden, Switzerland). All fawns and 30 females were tagged and therefore individually identifiable. Tagging of deer is carried out routinely by the owners each year and was not done for the purposes of this study. Females that had lost their tags (n = 4) were easily recognized using a combination of coat pattern and the individually distinctive tear patterns of

the ears following tag loss. All fawns were born between June 11 and 26. Adult females ranged in age from 2 to 10 years, but the age of individuals was not known.

Recording Procedure and Signal Acquisition

Calls were recorded according to Vannoni et al. (2005). We used a Praat 4.3 DSP package (P. Boersma and D. Weenink, University of Amsterdam, The Netherlands) to generate spectrograms (fig. 1*A*, Fast Fourier Transform [FFT] method, window length = 0.01 s, time step = 1,000, frequency step = 250, frequency resolution = 60 Hz, Gaussian window shape, dynamic range = 35 dB) of the vocalizations. Each call was visually inspected; vocalizations with high levels of background noise were rejected, and therefore good quality recordings were not available for all animals in each herd. We finally analyzed 574 calls from 12 fawns (13–112 calls for each individual) and 487 calls from 14 adult females (14–78 calls for each individual).

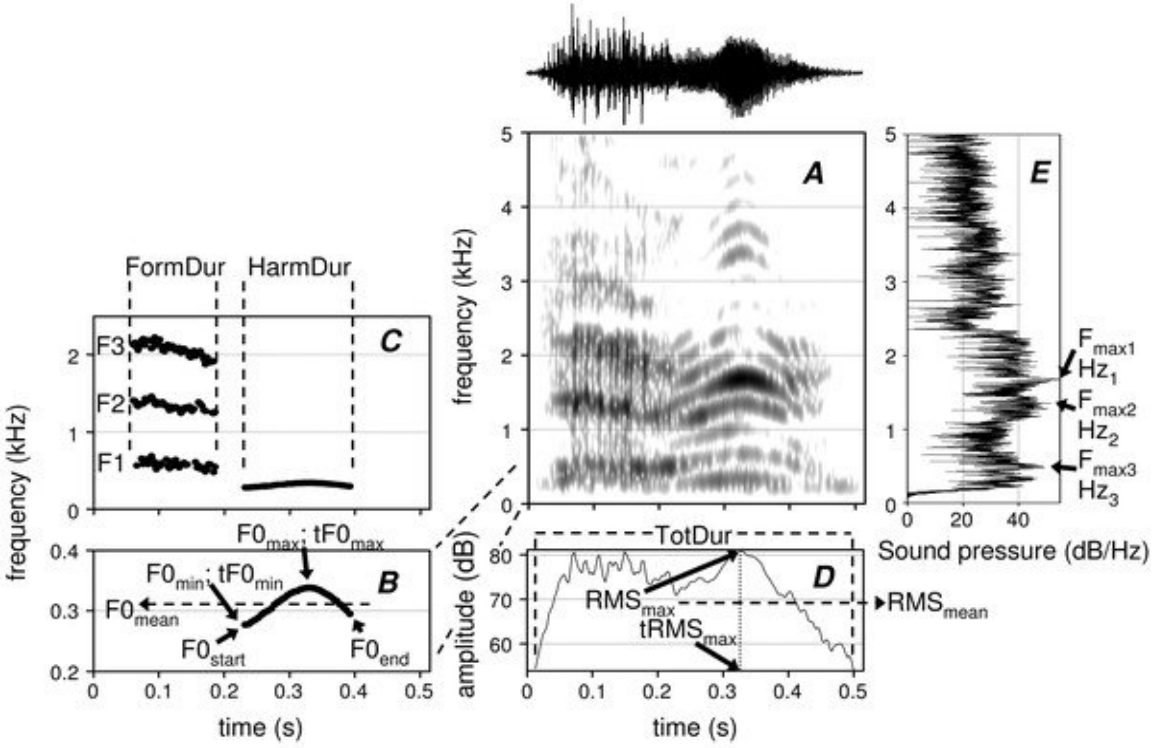

Figure 1: Analysis method of the acoustic parameters of adult female and fallow deer fawn contact calls, using an example of an adult female call. *A*, Spectrogram of the call (window length = 0.01 s, frequency resolution = 60 Hz, Gaussian window shape). *B*, Fundamental frequency profile. *C*, Formant pattern, estimated using linear predictive coding. *D*, Intensity contour. *E*, Average power spectrum, showing the energy distribution among frequencies

Acoustic Analyses

Time-varying numerical representations of the fundamental frequency (F0) for each call were produced using a pitch analysis (Sound: To Pitch (ac) command, time step = 0.001 s, Gaussian window) based on an autocorrelation algorithm associated with filtration of the signal (pitch floor = 350 Hz, pitch ceiling = 900 Hz for fawn calls; pitch floor = 150 Hz, pitch ceiling = 600 Hz for adult female calls) as described in Boersma (1993). We then used these frequency contours (fig. 1*B*) to measure the following F0 and temporal parameters: F0 mean ($F0_{mean}$), F0 initial ($F0_{start}$), F0 final ($F0_{end}$), F0 minimum ($F0_{min}$), F0 maximum ($F0_{max}$), time of F0 minimum ($tF0_{min}$), time of F0 maximum ($tF0_{max}$), total duration (TotDur) of the calls of fawns, and duration of the harmonic part (HarmDur) of the calls of adult females.

We used linear predictive coding analysis (Sound: To Formant [burg] command, time step = 0.001 s, maximum number of formants = 3, maximum formant = 1,800–2,600 Hz, window length = 0.025 s, preemphasis from 30 Hz; see Press et al. 1992) to estimate the formant frequencies (vocal tract resonances, fig. 1*C*) of adult female calls. We extracted the mean frequency of the first three formants (F1, F2, and F3) and measured the duration of the formant part (FormDur) for each call.

Using the intensity contour (fig. 1*D*, Sound: To Intensity command, minimum pitch = 350 Hz for fawn calls and 150 Hz for adult female calls, time step = auto) of the calls, we measured the maximum intensity (RMS_{max}), the mean intensity (RMS_{mean}), and the time when highest amplitude peak occurs ($tRMS_{max}$). Temporal parameters such as $tRMS_{max}$, FormDur, and HarmDur were divided by TotDur of the calls to express them in a relative way. Similarly, we also divided the temporal parameters related to F0 by HarmDur. Because the measurement of RMS_{max} and RMS_{mean} vary according to the distance between the calling animal and the microphone, and this distance was not always the same, we calculated the additional variable RMS_{max}/RMS_{mean} (RMS_{ratio}). Moreover, we used the intensity contour to calculate the total duration (TotDur) of the adult female calls.

For each call, we displayed average power spectra (fig. 1*E*; Sound: To Spectrum command, FFT) to quantify the distribution of the energy among frequencies. We identified the first three bands with energy peak (F_{max1}, F_{max2}, and F_{max3}) and measured their relative frequencies (Hz_1, Hz_2 and Hz_3). Then we calculated the percentage of occurrence of F0 of each harmonic and of each formant as the first three frequencies of highest amplitude.

Playback Experiments

We carried out playback experiments on 12 mother-fawn (6 males, 6 females) pairs that belonged to the Canton Zurich herd. The fawns were aged 52–73 days during playbacks, and therefore the hiding phase was over and they had been accompanying their mothers for at least 30 days. Each playback was performed when mothers and fawns had lost contact within the enclosure (~4 ha) and approximately 2 h had passed since the last suckling, and therefore all animals were motivated to respond. We observed the herd to determine when sucklings occurred. Sequences were played when mothers or fawns were lying down and were 15–50 m from the speaker. Calls were played back directly from a laptop computer that was connected to a battery-powered loudspeaker (Sound Projections Sound Machine SM-2, frequency response 65 Hz–16 kHz). Based on the observed natural occurrence of the reunion processes between mothers and their fawns, we did not perform more than two playback experiments/day. We tested all the fawns (6 females, 6 males) and half of the mothers twice during the study period, with 2 weeks between the two sessions to avoid habituation (McGregor et al. 1992). The remaining mothers were tested once. During playbacks, the observers and loudspeaker were concealed from view.

Each playback session consisted of two 30-s sequences played in random order: calls of own mother or own young and calls of an alien mother or an alien fawn. Playback sequences were separated by 2-min silence because this time interval allowed the animals to return to their previous activity before the next sequence was played. Each sequence was composed of 24 calls from the same individual, of which a minimum of 12 were different calls (for both treatments) to avoid pseudoreplication (Kroodsma et al. 2001; Wiley 2003). This number of calls represents a natural calling rate (48 calls/min; M. V. G. Torriani and A. G. McElligott, unpublished data). Because the call structure was likely to change as fawns grew, only calls recorded within 1 week before the experiments were included in the sequences played back to the mothers.

We used two cameras (Sony digital video camera, DCR-TVR 50E) to film the responses of two fawns (own and alien) or two mothers (own and alien) simultaneously, from 2 min before until 2 min after each playback. The reactions of the tested animals were classified as negative (0) if no behavioral change was detected and positive (1) if one of the following behaviors occurred: head turns with ears oriented toward the loudspeaker, call in reply, or movement toward the loudspeaker. When playbacks elicited the reunion between the

mother and fawn ($n = 6$), the session was interrupted, and the remaining sequence was not used in the statistical analysis. This explains the small sample size difference in the results of our playbacks.

Statistical Analysis

We investigated age and sex differences in fawn calls using a general linear mixed model (GLMM) procedure fitted with residual maximum likelihood estimation (REML, lme function; Venables and Ripley 2002). Individual identity was fitted as a random term, and age and sex were fitted as fixed effects. We considered two age classes of the same length (28–52 and 53–78 days old). We performed a multivariate GLMM including the F0-related parameters and a univariate GLMM for all the measured acoustic parameters. When the interaction between age and sex was not significant, we ran the analysis again, excluding the interaction term (Engqvist 2005). This analysis was performed in R for Windows version 2.0.1 (R Development Core Team 2004) with the packages "nlme" (Pinheiro et al. 2004) and "MASS" (Venables and Ripley 2002). All the other statistical analyses were performed using SPSS 11.5. Because F_{max1}, F_{max2}, and F_{max3} were ordinal measurements, for these parameters, age differences were tested using the Wilcoxon signed-ranks test and sex differences were tested using the Mann-Whitney *U*-test.

We used principal component analysis (PCA) to reduce the original data set of all measured variables of adult female and fawn calls to a smaller set of uncorrelated variables, retaining as much information as possible. The key variables that explained most of the variance were then included in the discriminant function analysis (DFA). With this method, we investigated whether there were differences that would allow discrimination between individuals and which acoustic parameter was most likely to code for individual identity. We separately analyzed 115 calls from 12 adult females (5–12 each, randomly selected from different recordings taken during July and August) and 140 calls of 12 fawns (8–12 each, randomly selected within the second age class).

In order to test the influence of the playback sequences on the behavior of mothers and fawns, we used a binary logistic regression model with the binary variable "response" or "no response." We then performed an ANOVA to investigate any difference in the duration of the positive reactions. All tests were two tailed and were considered significant if $P < .05$. Means were given with standard deviation (±SD).

RESULTS

Description of Calls

Contact calls of fawns were tonal (fig. 2*A*); their F0 ranged from 374.4 to 863.0 Hz (mean = 632.1 ± 54.9 Hz) and their duration from 0.05 to 0.38 s (mean = 0.17 ± 0.04 s). Adult females emitted three different calls: 69.3% of the recorded calls were composed of a noisy part first and a second part with harmonic structure (fig. 2*B*), 24.7% were tonal (fig. 2*C*), and 10.7% were completely noisy (fig. 2*D*). Calls of adult females were characterized by low F0 that varied from 152.7 to 579.1 Hz (mean = 365.0 ± 85.7 Hz). The mean frequencies of the first three formants were 539.0 ± 71.8 Hz for F1, 1,296.6 ± 66.9 Hz for F2, and 2,009.5 ± 166.0 Hz for F3. The call duration ranged from 0.14 to 0.62 s (mean = 0.35 ± 0.08 s).

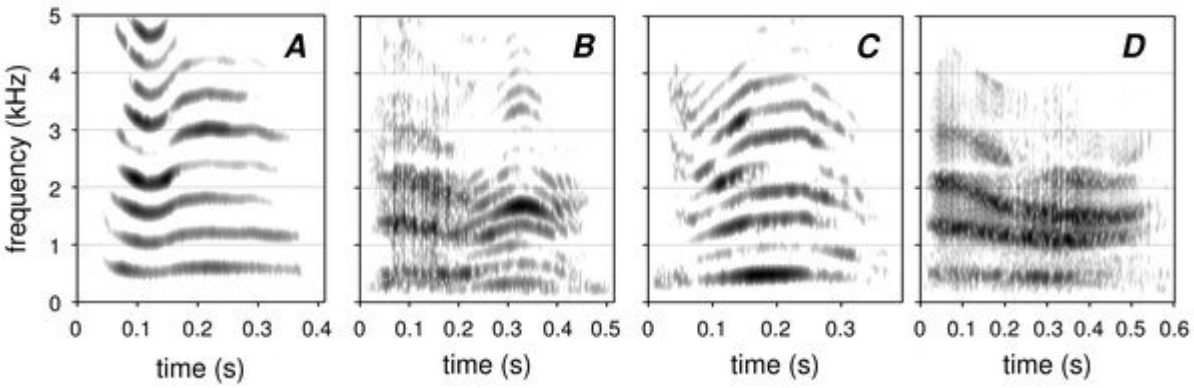

Figure 2: Narrow band spectrograms (window length = 0.01 s, frequency resolution = 60 Hz, Gaussian window shape) of typical contact calls of fawns with well-defined harmonics (*A*) and of adult females: call with a first noisy part and a second part with harmonic structure (*B*), tonal call (*C*); and noisy call (*D*).

Age and Sex Differences in Fawns

We found that as the fawns aged, the F0-related parameters became significantly lower (multivariate GLMM: $F = 231.39$, df = 1, 560, $P < .001$), and the duration of the calls became significantly longer (univariate GLMM: $F = 66.68$, df = 1, 560, $P < .001$; table 1). Intensity (RMS ratio) was significantly lower in the first than in the second age class (univariate GLMM: $F = 5.41$, df = 1, 560, $P = .020$). Parameters related to F0 modulation, intensity modulation, and energy distribution were not different in the two age classes (table 1). The F0-related parameters were significantly lower in males than in females (multivariate GLMM: $F = 5.08$, df = 1, 560, $P = .025$). In addition, there was no effect of sex on any of the other measured parameters (table 2).

Table 1. Descriptive statistics for the acoustic parameters of fallow deer fawn contact calls divided according to age.

		Age class 1	Age class 2	
	Variable	Mean ± SD	Mean ± SD	*P*
Duration (s)	TotDur	0.15 ± 0.04	0.20 ± 0.02	<0.001
Frequency (Hz)	$F0_{start}$	650 ± 67	581 ± 65	<0.001
	$F0_{end}$	638 ± 50	579 ± 39	<0.001
	$F0_{min}$	598 ± 64	540 ± 51	<0.001
	$F0_{max}$	699 ± 52	656 ± 43	<0.001
	$F0_{mean}$	653 ± 57	611 ± 46	<0.001
Modulation (%)	$tF0_{min}$	47 ± 30	50 ± 18	0.425
	$tF0_{max}$	47 ± 26	52 ± 15	0.084
	$tRMS_{max}$	37 ± 18	38 ± 9	0.328
Intensity	RMS_{ratio}	1.07 ± 0.02	1.08 ± 0.01	0.020
Energy peaks	F_{max1}	F0	F0	0.480
	F_{max2}	H1	H1	0.260
	F_{max3}	H3	H4	0.550

Note: Age class 1, 28–52 days; age class 2, 53–78 days. Age differences were examined using a general linear mixed model analysis. Measurements of the energy distribution (F_{max1}, F_{max2}, and F_{max3}) were tested using Wilcoxon signed-ranks test.

Table 2. Descriptive statistics for the acoustic parameters of fallow deer fawns contact calls divided according to sex and age.

		Age class 1		Age class 2		
		Females	Males	Females	Males	
	Variable	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	*P*
Duration (s)	TotDur	0.14 ± 0.06	0.15 ± 0.03	0.19 ± 0.02	0.20 ± 0.02	0.101
Frequency (Hz)	$F0_{start}$	665 ± 83	634 ± 49	610 ± 38	552 ± 77	0.035
	$F0_{end}$	639 ± 65	636 ± 36	597 ± 29	560 ± 41	0.034
	$F0_{min}$	601 ± 90	596 ± 30	565 ± 31	516 ± 58	0.009
	$F0_{max}$	710 ± 62	689 ± 44	671 ± 35	640.9 ± 48.7	0.371
	$F0_{mean}$	658 ± 74	649 ± 41	628 ± 33	593 ± 53	0.057
Modulation (%)	$tF0_{min}$	57 ± 37	39 ± 24	58 ± 16	45 ± 22	0.272
	$tF0_{max}$	45 ± 38	52 ± 12	50 ± 14	57 ± 16	0.504
	$tRMS_{max}$	42 ± 27	35 ± 5	37 ± 11	41 ± 8	0.405
Intensity	RMS_{ratio}	1.07 ± 0.04	1.07 ± 0.01	1.07 ± 0.00	1.08 ± 0.02	0.255
Peak Energy	F_{max1}	F0	H1	F0	F0	0.291
	F_{max2}	H2	H1	H1	F0	0.478
	F_{max3}	F0	H3	H4	H4	0.514

Note: Age class 1, 28–52 days; age class 2, 53–78 days. Sex differences were examined using a general linear mixed model analysis. Measurements of the energy distribution (F_{max1}, F_{max2}, and F_{max3}) were tested using Mann-Whitney *U*-test.

Individuality of Calls

We first performed a PCA on all the measured acoustic parameters for adult female and fawn calls separately. Based on the results of the PCA, we grouped all the measured parameters into four independent categories: duration, frequencies, time modulation, and energy. We then ran a second PCA within each category, and the comparison of the outcomes of both PCAs resulted in a final selection of four acoustic parameters that were included in the DFA: $F0_{mean}$, TotDur, $tF0_{min}$, and $tRMS_{max}$ for the calls of fawns; $F0_{mean}$, F3, TotDur, and $tF0_{min}$ for the calls of adult females.

Cross-validated DFA classified 81.7% of the adult female contact calls correctly, whereas only 32.1% of the fawn contact calls were correctly assigned (fig. 3). The $F0_{mean}$ was

the most individually distinctive parameter in both adult female and fawn calls, explaining 53.7% and 27.1% of the variability, respectively. In adult female calls, the mean frequency of the third formant alone explained 28.0% of the variance, the call duration alone 22.2%, and the time of F0 minimum alone 18.4%.

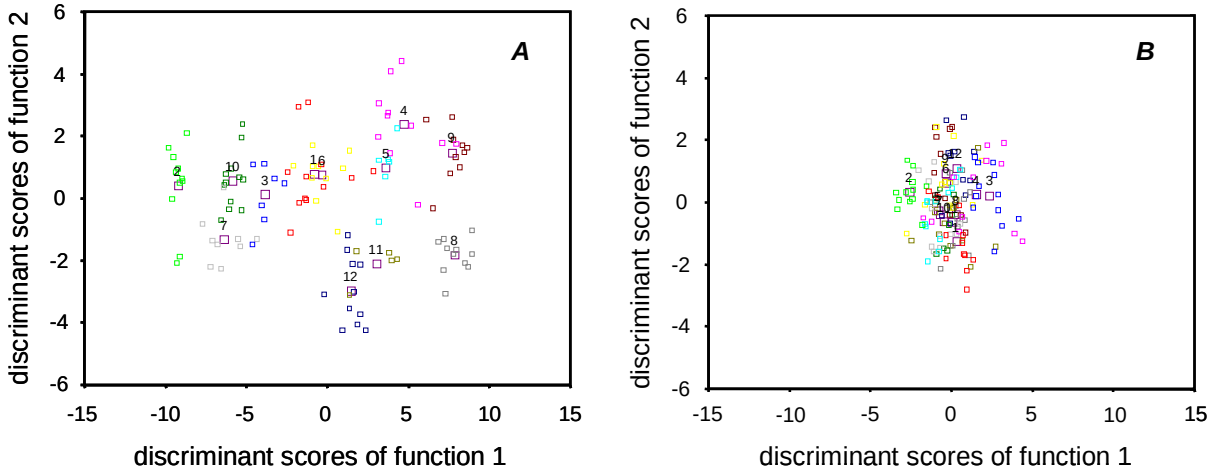

Figure 3: Discriminant function scores of adult female (*A*; *n* = 12) and fawn (*B*; *n* = 12) calls. Squares of the same color represent calls of the same individual.

Playback Experiments

Fawns reacted significantly more often to calls of their mothers than to calls of alien females (fig. 4*A*; χ^2 = 5.70, df = 1, 42, *P* = .02). Mothers reacted similarly to calls of their fawns and calls of alien fawns (fig. 4*B*; χ^2 = 0.49, df = 1, 32, *P* = .48). The interaction term for "receiver" (mother/fawn) and "playback type" (alien/own) was also significant (χ^2 = 6.08, df = 1, 74, *P* = .01). Thus, fawns could discriminate between own and alien mother contact calls, whereas mothers could not discriminate between own and alien fawns. We did not find significant differences in the duration of fawn reactions to own or alien mothers (ANOVA: *F* = 0.77, df = 1, 23, *P* = .40), or in the duration of mother reactions to own or alien fawns (ANOVA: *F* = 3.23, df = 1, 14, *P* = .10).

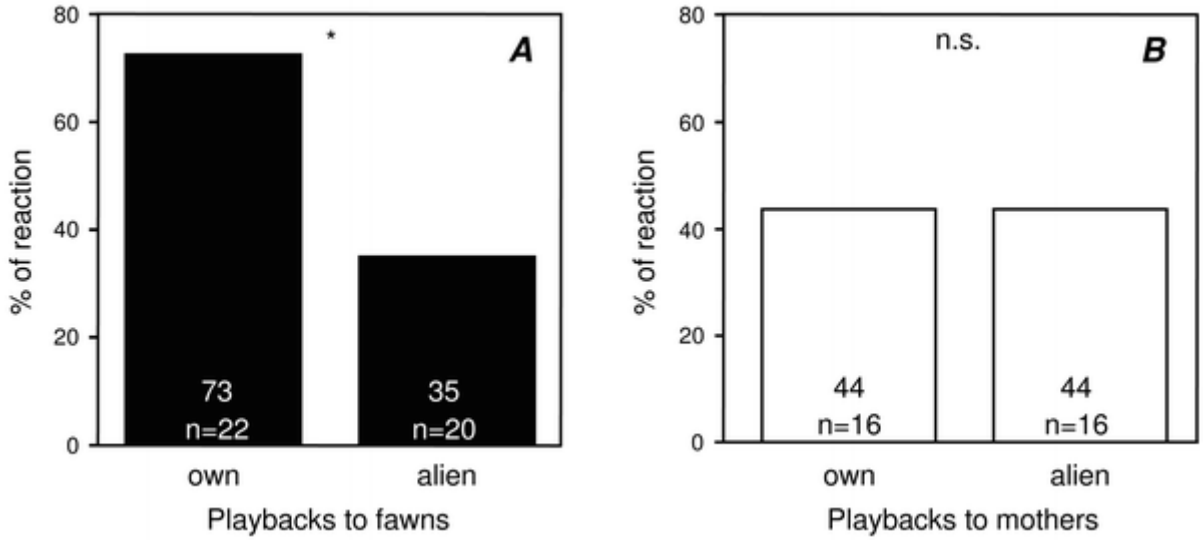

Figure 4: Results of playbacks of own and alien calls to (*A*) fawns and (*B*) mothers: the percentage of reactions to calls of the own mother or fawn and to calls of an alien mother or fawn. Comparisons between responses to own and alien calls were carried out using binary logistic regressions. Significance is shown above the bars: asterisk indicates $P < .05$; NS indicates not significant.

DISCUSSION

Our results show that only adult female fallow deer (and not fawns) have individualized contact calls and the fundamental frequency was the most individually distinctive parameter. Playback experiments confirmed that these calls were used by fawns for maternal recognition because they reacted more often to the calls of their own mothers than to the calls of alien females. We found that mothers did not recognize the contact calls of their own fawns. Our findings show that in an ungulate hider species, fawns are responsible for mother-offspring acoustic recognition. It is crucial for fawns to correctly recognize their mothers in order to receive milk, and to avoid leaving the hiding place in response to a female that is not its mother, during the early phase of life. At the same time, females gain direct fitness benefits because the production of individually distinctive calls allows the reunion process, and therefore survival of their offspring, to occur. This one-way recognition system persists into the age at which fawns join adults in the herd, as this is the age at which we carried out the playbacks. Studies of follower ungulates such as sheep (Searby and Jouventin 2003) and reindeer (Espmark 1971, 1974) found that both mother and offspring are able to recognize each other based on their contact calls. Together, these results suggest that the evolution of different antipredator strategies in young ungulates (following, hiding) also shaped the modalities of acoustic recognition between mothers and young. This appears to be independent

of phylogenesis, because although fallow deer and reindeer are more closely related than reindeer and sheep, the mother-offspring recognition of the latter two species is more similar.

The acoustic analysis showed that adult female calls were individually distinctive, with the fundamental frequency as the most distinctive parameter and temporal parameters less distinctive. Therefore, the acoustic recognition of fallow deer mothers by fawns is likely to be similar to that found in sheep (Searby and Jouventin 2003). The fundamental frequency is related to the length and thickness of the vocal folds in the larynx, and therefore, it is not surprising that this acoustic cue is highly individualized (Titze 1994; Charrier et al. 2002). We found that adult females produced three types of calls: tonal calls with a relatively high fundamental frequency, calls with a first noisy part and a second part with harmonic structure, and low-pitched noisy calls. In a noisy adult female call (fig. 2*D*), the formant frequencies decreased slightly during the call, thereby indicating a vocal tract elongation caused by a small lowering of the larynx (Fitch 1997; McElligott et al. 2006).

Fawn contact calls were not individually distinctive, and as the fawns grew, their calls became longer and deeper as the vocal folds lengthened and thickened and the larynx increased in size (Titze 1994). The changing call structure is most likely related to the lack of individuality, and together, these factors make recognition difficult. In fallow deer, males are born larger and grow faster than females, and these differences are reflected in the lower fundamental frequency related parameters in males (Birgersson and Ekvall 1997; this study). Our findings are consistent with those for red deer stags, in which the mean and maximum fundamental frequencies are higher on average in subadults than in adults (Reby and McComb 2003).

Our playbacks showed that fawns were able to discriminate between mother calls and those of alien females. However, fawns also sometimes reacted positively to calls of alien females. After the hiding phase, both allosucking and the stealing of milk from alien mothers are common in fallow deer (Birgersson et al. 1991; Pélabon et al. 1998; Roulin 2002). Therefore, it is not surprising that fawns sometimes react to the contact calls of alien mothers if it indicates that a suckling will occur because fawns only attempt to steal milk from alien mothers after they have begun to nurse their own fawns (Ekvall 1998). Mothers try to avoid misdirected maternal investment by checking the identity of their fawns using olfaction (Ekvall 1998). Fawns did not always respond to own mother calls. The presence of a visual

cue (e.g., an approaching mother) could also act as a stimulus for the fawns and perhaps enhance the positive reactions to own mother calls (Shillito-Walser et al. 1981).

The playbacks to mothers showed that they did not recognize their own fawns using contact calls. During the hiding phase, the mothers call the fawns to initiate the reunion process, whereas fawns are largely silent (Gilbert 1968; Braza and San Josè 1988). There is little opportunity for mothers to learn the calls of their offspring because of the limited periods that they spend together. Moreover, our results have shown that there is little individuality in the calls of fawns, even at the older ages, when they have joined adults in a herd and they vocalize more frequently. It is also unlikely that the results of our playbacks to mothers could have been due to mothers ignoring the calls of their own fawns, because they were carried out when fawns were 7–10 weeks old. Females do not stop nursing their young until they are several months older than this (Birgersson and Ekvall 1997). Our results suggest that the environmental conditions (closed vs. open habitats) that influence predator avoidance strategies (hiding/following) in turn affect parent-offspring contact and recognition mechanisms and the development of the vocal apparatus in young mammals. In terms of the vocal apparatus, we mean the ability or inability (in the case of ungulate hiding young) to produce individually distinct vocalizations.

The vocal characteristics of fawns change as they grow, and therefore, it is crucial for fawns to recognize their mothers from the more stable characteristics of their adult calls. The complex mutual vocal recognition systems found in mammals that live in large mixed groups of adults and offspring (Insley 2000, 2001; Charrier et al. 2001, 2002; Searby and Jouventin 2003) may have evolved because of the potential for identity confusion at early ages in these species. In most other mammals, including hiding ungulates, a less complex vocal recognition system along with olfaction seems to be enough to ensure that mothers and offspring can recognize one another effectively and communicate. Future studies of parent-offspring vocal recognition in mammals and other species should focus on determining whether there are asymmetries in recognition that depend on the dynamics of how parents interact with offspring in early life.

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CHAPTER 3

Retraction of the mobile descended larynx during groaning enables fallow bucks (*Dama dama*) to lower their formant frequencies

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Retraction of the mobile descended larynx enables fallow bucks (*Dama dama*) to lower their formant frequencies

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ABSTRACT

A permanently descended larynx is found in humans and several other species of mammals. In addition to this, the larynx of species such as fallow deer is mobile and in males it can be retracted during vocalization. The most likely explanation for the lowered retractable larynx in mammals is that it serves to exaggerate perceived body size (size exaggeration hypothesis) by decreasing the formant frequencies of calls. In this study, we quantified for the first time the elongation of the vocal tract in fallow bucks during vocalization. We also measured the effect of this vocal tract length (VTL) increase on formant frequencies (vocal tract resonances) and formant dispersion (spacing of formants). Our results show that fallow bucks increase their VTL on average by 52% during vocalization. This elongation resulted in strongly lowered formant frequencies and decreased formant dispersion. There were minimal changes to formants 1 and 2 (−0.91 and +1.9%, respectively) during vocal tract elongation, whereas formants 3, 4 and 5 decreased substantially: 18.9, 10.3 and 13.6%, respectively. Formant dispersion decreased by 12.4%. Formants are prominent in deer vocalizations and are used by males to gain information on the competitive abilities of signallers. It remains to be seen whether females also use the information that formants contain for assessing male quality before mating.

INTRODUCTION

Recent research has shown that a descended larynx in mammals is more widespread than previously believed, and is not restricted to humans (Fitch & Reby, 2001; Weissengruber et al., 2002). Humans *Homo sapiens*, for example, have a permanently descended larynx; it moves from its high intranarial location down into the throat between 3 months and 3 years of age. There is a secondary descent of the larynx that occurs at puberty and is restricted to males (Fitch & Giedd, 1999). In addition to being descended, the larynx of red deer stags *Cervus elaphus* and fallow bucks is mobile and can be retracted even further during vocalization by the sterno-thyroid and sterno-hyoid muscles. This increases the vocal tract length (VTL) and results in lower formant frequencies (vocal tract resonances) and formant dispersion (spacing of formants) (Fitch, 2000a; Fitch & Reby, 2001; Weissengruber et al., 2002; Reby & McComb, 2003a,b).

There are a number of hypotheses that have been used to explain the evolution of the permanently descended larynx. Lieberman, Klatt & Wilson (1969) suggested that a low larynx allows humans to create a wider range of vocal tract shapes, and thus more varied and distinctive speech sounds than other mammals. Thus, lowering of the larynx has been considered a key point in the evolution of the spoken human language. However, there are many non-speaking species, which possess a descended and/or retractable larynx (Sonntag, 1921; Fitch, 2000a; Fitch & Reby, 2001; Weissengruber et al., 2002). Other hypotheses propose that the laryngeal lowering was a non-adaptive by-product of upright posture or it could be adaptive for mouth breathing during extreme physical challenge (Dubrul, 1976; Lieberman, 1984). Moreover, laryngeal lowering could also increase the effectiveness with which calls propagate through the environment by enhancing the low-frequency components (Morton, 1975; Michelsen, 1978; Wiley & Richards, 1982).

The evolution of the descended larynx, particularly in males of several independent lineages (e.g. cats, deer, humans), suggests that it could be related to the signalling of competitive abilities (Hauser, 1996; Fitch & Reby, 2001; Seyfarth & Cheney, 2003). In humans, macaques *Macaca mulatta* and dogs *Canis familiaris*, body size and VTL are positively related to one another (Fitch, 1997, 2000b; Fitch & Giedd, 1999; Riede & Fitch, 1999). VTL is also directly related to formant frequencies (Fitch & Reby, 2001). Therefore, Fitch & Reby (2001) proposed that the descended larynx in deer serves to give receivers an

exaggerated impression of body size (size exaggeration hypothesis) by decreasing the formant frequencies and formant dispersion of calls. The resulting change in perceived body size could have increased the chances that males would attract mates and/or deter competitors. Nevertheless, the minimum formant frequencies, achieved when the larynx is fully pulled down to the sternum, still provide an honest indication of body size (Reby & McComb, 2003a). This is because the sternum acts as a morphological constraint, beyond which further retraction of the larynx is not possible. Additional support for the size exaggeration hypothesis comes from playback experiments on red deer males. Red deer males use formants to assess other males during roaring contests and can adjust their own formant frequencies in relation to those that they hear (Reby et al., 2005).

Fallow bucks only vocalize during the breeding season. They start groaning in late September and produce a low-pitched, stereotyped and repetitive call ranging from 20 to 8000 Hz (Reby et al., 1998; McElligott, O'Neill & Hayden, 1999; Fitch & Reby, 2001; Vannoni, Torriani & McElligott, 2005). One groan lasts on average 0.3–0.5 s and is composed of regularly spaced pulses, which are produced by vibrations of the vocal cords (Reby et al., 1998). Fallow bucks modify their vocal tract during calling by pulling the larynx down towards the sternum (Fitch & Reby, 2001).

Male deer that are able to retract their larynges further than average, and thus lower their formant frequencies and increase their perceived body size, would have advantages in intrasexual competition and intersexual advertisement (Fitch, 2002; Reby et al., 2005). In this study, we quantify for the first time the elongation of the vocal tract in fallow bucks during vocalization. We also measure the effect of this VTL increase on formant frequencies and formant dispersion.

METHODS

Study site and study population

This study was carried out in Phoenix Park, Dublin, Ireland. It is a large enclosed city park (709 ha, 20% open woodland, 80% pasture), situated 2.4 km west of the centre of Dublin (53°22'N, 6°21'W).

Audio and video recordings

We carried out audio and video recordings of mature, groaning fallow bucks (≥4 years old; McElligott et al., 1998) from 12 October to 29 October 2003. We used a directional microphone (Sennheiser MKH-70; Old Lyme, CT, USA) plugged into a digital video camera (Sony, DCR- TRV50E; Tokyo, Japan). In other cases we recorded the audio and video sequences separately with the video camera and a digital audio tape recorder (Sony, TCD-D100) connected to the directional microphone. The recorded animals were between 20 and 100 m away from the microphone and at an angle of c. 90° to the video camera. After each recording session, we recorded a meter stick at the same location as the male (n=7 males). Distances were measured using a Leica rangefinder (Leica Camera AG, Solms, Germany). During picture analysis, we used this recorded meter stick to obtain a suitable reference for converting the pixels into centimetres.

Body measurements

On 23 September, one male was caught and some body measurements were taken (for details of the capture method, see McElligott et al., 2001). These were the distance from the pre-orbital gland to the tip of the nose, on both sides of the head. This facial measurement was also taken from two males that died just after the breeding season, in November. The facial measurements also served as references, which were crucial to convert pixels into centimetres, for the three remaining males (total n=10; see Results).

VTL measurements and changes during groaning

All pictures needed for VTL measurements were extracted from video sequences using Pinnacle Studio Version 8. We measured the position of the larynx on the pictures using Scion Image Beta Version 4.0.2 (available from http://www.scioncorp.com). VTL was measured as the distance from the tip of the snout to the larynx. We converted this value from pixels into centimetres using the meter stick or the facial measurements (Fitch & Reby, 2001). The minimum VTL was measured three times on three different pictures for each male (n=10). Therefore, the minimum VTL (when the larynx was in the resting position) was calculated as the mean of nine measurements. It should be more or less a fixed value for a male, because this is when the males are not groaning and the larynx is in the resting position. We also measured the maximum VTL (when the larynx was retracted) for each groan in the same way. Finally, it was possible to measure the distance from the tip of the snout to the sternum for four males, and again we used the mean of nine measurements.

We measured the VTL at the pre-groan stage (when the males were not groaning), at the onset of vocalizing, and when the larynx was fully pulled down. We compared the change in formant frequencies and formant dispersion over those three stages ('three-frame groan analysis'; Fig. 1). We used the frequencies and VTL measurements of one to seven groans from nine males (four males, four groans; two males, five groans; three males, one, three and seven groans, respectively).

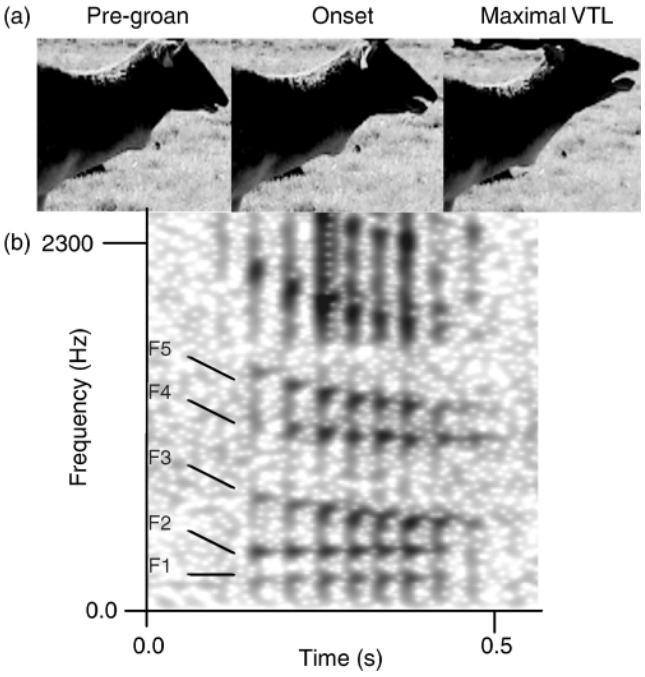

Figure 1 (a) Photos taken from videotape of a fallow buck groaning once [vocal tract length (VTL) increase over the three stages: pre-groan, onset and maximal VTL]; (b) spectrogram of the emitted groan, showing decreasing formant frequencies of five clearly detectable formants during groaning.

Acoustic analysis

We extracted the audio files from the video sequences using Pinnacle Studio Version 8. The sample rate of the extracted original recordings was 48 000 Hz. Formant frequencies were measured using linear predictive coding analysis (Press et al., 1992) with Praat 4.2 (available from Paul Boersma & David Weenink, http://www.praat.org). We used formants 3, 4 and 5 (F_3–F_5) for our detailed analyses. The first two formants (F_1, F_2) were usually flat and these are simply reported. It is assumed that the F_1 and F_2 frequencies are absorbed by the vocal tract and that this is the reason for the flat appearance in the spectrogram (Fig. 1; W. T. Fitch, pers. comm.). The parameters were set as follows: maximum formant, 2000 Hz; maximum number of formants, 6–14; window length, 0.1 or 0.2 s; time step, 0.04 s. We calculated formant dispersion (D_f) according to Reby & McComb (2003a).

Relationship between VTL and formants (F_3–F_5, formant dispersion)

We carried out a framewise analysis of formant frequencies, formant dispersion and VTL during groaning (frame window=0.04 s). We used measurements of 1–11 groans from nine males (three males, six groans; two males, eight groans; four males, one, five, seven and 11 groans, respectively).

Statistical analysis

All statistical analyses were performed using R for Windows version 2.0.1 (R Development Core Team, 2004): the packages 'nlme' (Pinheiro et al., 2004) and 'MASS' (Venables & Ripley, 2002). All tests are two-tailed and factors were considered to have a statistically significant influence if $P<0.05$. All means are given with standard errors. To determine the relationship between increasing VTL and formant frequencies, we used a linear mixed effects model procedure fitted with residual maximum likelihood estimation (REML, lme function; Venables & Ripley 2002). The number of groans nested within individual identity was fitted as a random term. In this way, we controlled for repeated sampling within individuals. Formant frequencies and formant dispersion were fitted as fixed effects. We checked our data to verify that the assumptions of residual normality and variance homoscedasticity were satisfied. Good video recordings were available for 10 males (results in Table 1). However acoustic analyses were conducted for nine males because there were no good quality sound recordings for one male (remaining results).

RESULTS

Change in VTL during groaning

The data for mean minimum VTL, maximum VTL, absolute VTL increase and percentage increase of VTL for 10 males are given in Table 1. These were measured from the images of the vocalizing males. The overall mean minimum VTL was 32.2±1.5 cm, range 28.8–34 cm. The overall mean maximum VTL was 49.0±2.8 cm, range 44–59 cm. The males increased their VTL on average by 17.5±2.9 cm, range 13.8–20.3 cm (Table 1). This represents an overall percentage increase in VTL of 52.0±6.7%, range 42.9–61.1% (Table 1).

For the four males for which we could measure the distance from the tip of the snout to the sternum, the overall mean was 75.1±6.6 cm, range 63–85 (w433=66.3±2.1 cm, g477=73.8±1.9 cm, y717=81.1±3.8 cm, y736=79.2± 2.7 cm). Therefore, the maximum VTL

that these males attained represented 68.1±3.1% of the distance between the tip of the snout and sternum.

Table 1 Minimum vocal tract length (VTL), maximum VTL, absolute VTL increase and percentage VTL increase for each male (*n*=10)

Male	Age (years)	Minimum VTL	Maximum VTL	VTL increase	VTL increase (%)
b309	5	32.0 ± 0	46.7 ± 0.58	18.7 ± 0.58	45.9
y736	6	34.0 ± 0.5	54.3 ± 4.15	20.3 ± 4.15	59.7
y717	6	33.6 ± 1.51	53.3 ± 1.26	19.7 ± 1.26	58.6
y746	6	32.2 ± 1.09	50.0 ± 0	17.8 ± 0	55.3
y693	6	32.1 ± 0.78	48.4 ± 2.07	16.3 ± 2.07	50.8
y747	6	32.9 ± 0.33	48.0 ± 1.5	15.1 ± 0	45.9
w433	7	31.2 ± 2.17	48.0 ± 1.83	16.8 ± 1.83	53.8
w591	7	28.8 ± 0.97	46.4 ± 1.67	18.1 ± 1.67	61.1
w504	7	32.2 ± 0.67	46.0 ± 1.41	13.8 ± 1.41	42.9
g477	8	33.3 ± 1	48.5 ± 1	15.2 ± 1	45.6

Relationship between VTL and formants (F_3–F_5, formant dispersion)

For the nine males for which we had good sound recordings, the VTL increased by 54.0% (Table 2). There were minimal changes in F_1 (−0.91%) and F_2 (+1.9%) during vocal tract elongation, whereas F_3 decreased by 18.9%, F_4 by 10.3% and F_5 by 13.6% (Table 2). Formant dispersion decreased by 12.4% (Table 2).

Table 2. Vocal tract length (VTL), frequencies of the first five formants (F1-F5) and formant dispersion (D_f), as the larynx descends during vocalising. A, VTL at rest (pre-groan); B, minimum VTL during the groan (measured at the onset of vocalising); C, maximum VTL during the groan (*N* = 37 groans, *N* = 9 males).

	VTL (cm)	Formant frequencies (Hz)					
		F_1	F_2	F_3	F_4	F_5	D_f
A	32.2 ± 2.2	—	—	—	—	—	—
B	32.9 ± 2.0	220.4 ± 22.1	404.8 ± 31.7	708.5 ± 44.9	1183.1 ± 48.2	1458.4 ± 105.5	319.8 ± 16.5
C	49.6 ± 3.7	218.4 ± 20.6	412.4 ± 19.8	574.7 ± 50.1	1061.7 ± 37.0	1259.7 ± 46.1	280.0 ± 9.9

The framewise analysis revealed a strong negative relationship between formant frequencies and the location of the larynx (VTL), and also between formant dispersion and VTL. F_3–F_5 and formant dispersion decreased significantly with increasing VTL (linear mixed effect model: F_3, $F_{1,536}$=2448.5; F_4, $F_{1,547=}$1913.7; F_5, $F_{1,540}$=1792.3; formant dispersion, $F_{1,523}$=2838.2; P<0.0001 in all cases). The negative relationships between formant frequencies, formant dispersion and VTL are illustrated using one groan from one male in Fig. 2.

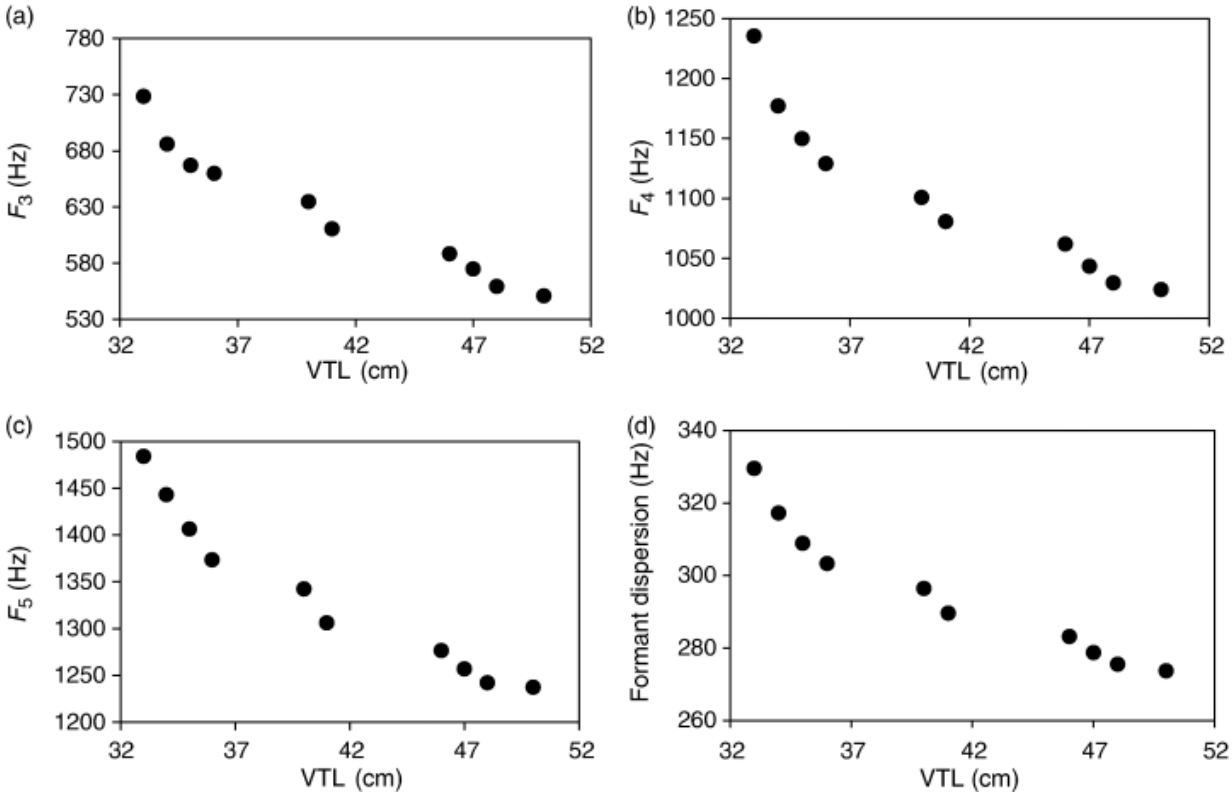

Figure 2 Relationship between increasing vocal tract length (VTL) and (a) formant 3, (b) formant 4, (c) formant 5 and (d) formant dispersion. The example is taken from a single groan of one male (*n*=10 frames).

DISCUSSION

Our results show that fallow bucks increase their VTL on average by 52% during vocalization, and the maximum VTL that some males attained was 65% of the distance between the tip of the snout and sternum. This elongation resulted in lowered formants (vocal tract resonances) and consequently decreased formant dispersion (spacing of formants). Most evidence suggests that the evolution of the descended larynx in cervids and other mammals occurred through 'size exaggeration', whereby males attempt to exaggerate their perceived

body size by modifying the phonic structure of their calls (Fant, 1975; Fitch & Reby, 2001; Reby & McComb, 2003a,b). Recent research has also revealed that red deer stags use the formant frequencies of roars for assessing competitors during intrasexual competition and can adjust their own formants in relation to those of perceived rivals (Reby et al., 2005).

Formants and formant dispersion are key features of calls because they are related to the length and shape of the supralaryngeal vocal tract and are related to body size (Fitch, 1997, 2000b; Riede & Fitch, 1999). Body size in turn is often related to male competitive abilities and reproductive success (Andersson, 1994; McElligott et al., 2001). The role of formants in intersexual advertisement, and possibly mate choice in deer, remains to be studied (Reby et al., 2005). However, there is already evidence from humans showing that females prefer lower formant frequencies, because they are indicative of sexual maturity in males (Feinberg et al., 2005). In humans, lower fundamental frequencies and/or increasing apparent VTLs of males increased female ratings of masculinity, size and age of the speaker (Feinberg et al., 2005). In addition, Reby & McComb (2003a) showed that there was a strong relationship between the minimum formant frequencies (reached during the part of the roar when the larynx is most fully retracted towards the sternum) and reproductive success in red deer. This association of higher reproductive success with formant frequencies is likely to be achieved through both intersexual advertisement and intrasexual competition.

Increasing the VTL during vocalization results in lower formant frequencies (Fitch & Reby, 2001; this study). In general, lower frequency sounds travel further than higher frequency ones, because higher frequencies are affected more by the environment, and therefore the evolution of the mobile descended larynx could have resulted from the need to increase the effectiveness of sound propagation over longer distances (Morton, 1975; Michelsen, 1978; Wiley & Richards, 1982; Fitch & Reby, 2001). However, when calls are emitted close to the ground, as is the case in fallow and other deer species, there is a peak in attenuation in the 300–800 Hz range (Morton, 1975; Marten & Marler, 1977; Fitch & Reby, 2001). This is the frequency range that is also produced by the lowering of the larynx, and therefore increasing the distances over which calls are heard cannot explain its evolution. The movement of the larynx during vocalization and the resulting lowered formants may in fact reduce sound propagation (Fitch & Reby, 2001).

The increase in VTL of fallow bucks during calling, at 52% (this study), is far less than that of red deer stags (c. 100%;Fitch & Reby, 2001). In an earlier review it was noted that red deer stags usually pull down the larynx to its maximum level at the sternum for almost all roars. By contrast, in our study, we found that fallow bucks pull the larynx to 65% of the distance between the tip of the snout and the sternum. This was also noted in an earlier review by Reby & McComb (2003b). Roaring in red deer also begins when the larynx is already pulled down by 37% (Fitch & Reby, 2001), whereas groaning in fallow deer begins immediately with laryngeal descent (see Table 2). These important differences probably result from the very different rates at which these two species vocalize. Whereas fallow bucks commonly achieve groaning rates of 60 and over per minute, the maximum roaring rate of red deer stag is c. 8 min^{-1} (Clutton Brock & Albon, 1979; McElligott & Hayden, 1999, 2001). Therefore, it suggests that achieving very high calling rates is more important for fallow deer than always lowering the larynx to its maximum level at the sternum, as in red deer.

Elongating the vocal tract in order to exaggerate their perceived body size would initially have been advantageous for males when this trait first started to evolve. However, once all males can lower the larynx, the net benefit of this adaptation would be eliminated (Fitch & Reby, 2001). Both fallow bucks and red stags compete intensively for access to mating opportunities during the breeding season, and as a result they lose a great deal of body condition (Yoccoz et al., 2002; McElligott et al., 2003). The loss in body condition, particularly towards the end of the breeding season, could also affect the ability of a male to retract the larynx during vocalization, because it is likely that current energy reserves also affect the muscles responsible for retraction. Therefore, the relative abilities of males to reduce the formant frequencies of calls could potentially provide information on current body condition and competitive abilities. It is already known that fallow bucks are able to detect changes in the current condition of rival males (McElligott et al., 1998), and vocalizations are likely to be one of the main routes through which this information is obtained.

This is the first time that the increase in the VTL during vocalization for fallow bucks has been measured, and its subsequent effect on the formant frequencies quantified. It is clear that formants form a very important component of the phonic structure of vocalizations that are used by males of some mammals to gain information on signallers (Fitch & Reby, 2001; Reby & McComb, 2003a; Reby et al., 2005). Future research should examine if females also use the information that is contained in formants.

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CHAPTER 4

Individual acoustic variation in fallow deer (*Dama dama*) common and harsh groans: a source-filter theory perspective

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Individual acoustic variation in fallow deer (*Dama dama*) common and harsh groans: a source-filter theory perspective

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ABSTRACT

Mammals are able to distinguish conspecifics based on vocal cues, and the acoustic structure of mammal vocalizations is directly affected by the anatomy and action of the vocal apparatus. However, most studies investigating individual patterns in acoustic signals do not consider a vocal production-based perspective. In this study, we used the source-filter model of vocal production as a basis for investigating the acoustic variability of fallow deer groans. Using this approach, we quantified the potential of each acoustic component to carry information about individual identity. We also investigated if cues to individual identity carry over among the two groan types we describe: common and harsh groans. Using discriminant function analysis, we found that variables related to the fundamental frequency contour and the minimum frequencies of the highest formants contributed most to the identification of a given common groan. Common groans were individually distinctive with 36.6% (53.6% with stepwise procedure) of groans assigned to the correct individual. This level of discrimination is approximately six times higher than that predicted by chance. In addition, univariate ANOVAs showed significant inter-individual variation in the minimum formant frequencies when common and harsh groans were combined, suggesting that some information about individuality is shared between groan types. Our results suggest that the sound source and the vocal tract resonances act together to determine groan individuality and that enough variation exists to potentially allow individual recognition based on groans.

INTRODUCTION

The acoustic structure of vocalizations is usually variable and can be affected by factors such as species identity, individual identity, social context, and phenotypic traits of the vocalizing animal (Fischer et al. 2002; Gerhardt & Huber 2002; Reby & McComb 2003a; Blumstein & Munos 2005). Individual variation in temporal and/or spectral features of acoustic signals is a pre-requisite for individual recognition. In situations in which vocal recognition is useful, selection may favour more stereotyped individual calls and the ability to perceive the discriminatory cues (Aubin et al. 2000; Insley 2001).

Individual vocal recognition may be adaptive for sexual selection, and influence male–male competition and female choice (Davies & Halliday 1978; McComb 1991). The ability to distinguish between stranger and familiar males based on individually distinctive calls can help prevent unnecessary contests (Stoddard 1996). Moreover, females may become familiar with individual vocal differences and preferentially mate with familiar males who can afford higher energy courtship displays (East & Hofer 1991; Zimmerman & Lerch 1993). Therefore, it could be advantageous for vocalizing animals to broadcast information about individual identity.

Individual recognition based on vocal signatures is common in mammals (Rendall et al. 1996; Sayigh et al. 1998; Frommolt et al. 2003). The presence of individual characteristics in mammal vocalizations is not surprising because the individual morphology of their vocal apparatus directly influences the spectral structure of the emitted signal (Fitch & Hauser 1995, 2003; Riede et al. 2005; McElligott et al. 2006). However, other than in humans (*Homo sapiens*), few studies have examined the individuality of vocalizations in relation to their biomechanical modes of production (Rendall et al. 1998; Soltis et al. 2005; Reby et al. 2006).

The source-filter theory of voice production (Fant 1960) predicts that the spectral structure of most mammal vocalizations results from the linear combination of two independent mechanisms. First, the source signal is generated by the vibration of the vocal folds during the passage of air in the glottis and characterizes the fundamental frequency (and its associated harmonics) of the vocalization. Then the source signal passes through the supra-laryngeal vocal tract where the resonance properties of the vocal tract cavities act as a filter and determine the amplification of specific frequencies called 'formants'. The formants finally radiate out through the mouth and nostrils into the environment. Because fundamental frequency and formant frequencies vary independently, one or both may provide receivers with information on caller identity (Rendall et al. 1998; Bachorowski & Owren 1999).

Both sexes of some deer species are known to produce individually distinctive calls (Espmark 1974; Vaňková & Málek 1997; Reby et al. 1998, 1999a; Torriani et al. 2006; Reby et al. 2006). The fallow deer is a highly polygynous and size dimorphic deer species (McElligott et al. 2001). While mature females may vocalize throughout the year using contact or alarm calls, mature males only produce their sexually selected groans during the breeding season and are otherwise silent. In the northern hemisphere, males start to vocalize towards the end of September and continue until early November. The vast majority of matings occur during the latter half of October and therefore some males begin to vocalize approximately 3 wk before the first matings occur (McElligott et al. 1999). The most common groan consists of a short, low-pitched and stereotyped vocalization characterized by a train of regularly spaced pulses (Reby et al. 1998). Vocalizations may be used by males to signal their presence to potential mates and to identify and localize competing males (McElligott & Hayden 1999). Such a role for groaning suggests the existence of recognition cues for individuality. A previous study showed that fallow deer common groans contain information on individuality (Reby et al. 1998), but the factors responsible for inter-individual acoustic variation have not been explored in detail and the relative importance of different call features for achieving individuality remains unknown. Fallow deer males also produce a more noisy and harsh groan, and the acoustic structure of this call has never been described (E. Vannoni & A. G. McElligott, personal observation). Because the production of various call types may rely on similar actions of the vocal folds and vocal tract of a specific individual, the acoustic cues to individuality originating from these processes may carry over across the vocal repertoire of the species (Rendall et al. 1998; Reby et al. 2006).

In this study, we investigated the individual acoustic variation of fallow buck groans in relation to the principles of the source-filter theory of sound production. We extracted and measured the spectral parameters of common and harsh groans and compared the formant pattern of the two types of call. Furthermore, we determined whether the acoustic structure of common groans varies according to individual identity, and the relative contribution of each feature in discriminating between different individuals. We finally determined if the acoustic individuality found in the formant pattern of common groans holds across harsh groans.

METHODS

Study Site and Subjects

The study was carried out on a herd of European fallow deer in Phoenix Park (709 ha, 80% pasture, 20% woodland; 53°22'N, 6°21'W), Dublin, Ireland during five breeding seasons between 1997 and 2004. All males used in this study were of known age, had ear tags and were therefore individually distinguishable.

Sound Recording and Signal Acquisition

Vocalizations were recorded using a Sony digital audio tape recorder DAT-TCD D100 connected to a Sennheiser MKH 70 directional microphone. Recordings were performed between dawn and sunset when a male was groaning at a distance of 10–50 m from the microphone.

Vocalizations were transferred to a computer at a sampling frequency of 22.05 KHz using Avisoft SASLab Pro 4.36 and saved in WAV format, at 16-bit amplitude resolution (Vannoni et al. 2005). As most of the recordings did not contain energy above 8 KHz, they were down-sampled to 16 KHz for a better frequency resolution. Narrow-band spectrograms of common and harsh groans (Fig. 1, FFT method, window length = 0.03 s, number of time steps = 1000, number of frequency steps = 250, frequency resolution = 20 Hz, Gaussian window shape, dynamic range = 35 dB) were generated using Praat 4.3.27 DSP package (P. Boersma & D. Weenink, University of Amsterdam, Amsterdam, The Netherlands). Each groan was visually inspected and vocalizations with high levels of background noise were rejected.

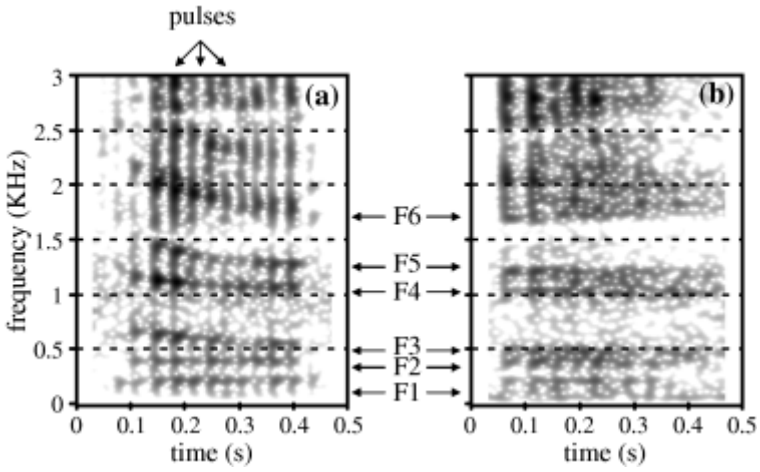

Fig. 1: Narrow band spectrograms of (a) a 'common' groan; and (b) a 'harsh' groan. The pulses and the first six formants are indicated.

During the breeding season, fallow deer males feed very little and lose approximately 26% of their body weight (McElligott et al. 2003), and there is some evidence from primates and deer that the acoustic structure of vocalizations can be affected by exhaustion (McComb 1988; Fischer et al. 2004). We therefore analysed recordings taken between October 9 and October 19 when only about 10% of the total number of matings had usually occurred (McElligott et al. 2001) and the majority of agonistic interactions among males were non-contact displacements (McElligott et al. 1998). This minimized the possibility that variation in the phonic structure of groans could have occurred due to exhaustion of the animals.

Vocalizations produced within the same bout are more likely to be acoustically homogeneous than vocalizations emitted in different bouts (Reby et al. 1999b), and therefore not statistically independent. To minimize this problem, the majority of the common groans (82%) were randomly selected from different bouts and the remaining (18%) groans belonged to long vocalization bouts but were never consecutive.

Acoustic Analyses

Common groans are short and low-pitched vocalizations, characterized by a train-pulse structure (Fig. 1a). The pulses represent the vibrations of the vocal folds and determine the fundamental frequency of the call. Fundamental frequency (F0) is defined as the inverse of the interpulse interval and this can be measured as the distance between consecutive pulse onsets (Fig. 2a). Distances between pulses were measured automatically from the envelope (amplitude vs. time) of the signal by using Pulse Train Analysis (Fig. 2b; method = rectification + exponential decay; pulse detection = peak search with hysteresis; time constant = 1 ms; threshold = 0.1 V; hysteresis = 16–19 dB) in Avisoft SAS Lab-Pro 4.32. We calculated the values of the F0 along the groan and then averaged these values to obtain the mean F0 ($F0_{mean}$). Because all groans showed at least a modest frequency inflection, the minimum and the maximum F0 ($F0_{min}$ and $F0_{max}$) were also included in the analysis. From the envelope of the signal we also calculated the number of pulses (pulses) and the duration of the groan (duration) as the distance between the onset of the first pulse and the end of the last one (Fig. 2b). Finally, we quantified the variation of F0 along the call using a measure of F0 perturbation, known as Jitter (Titze 1994). Jitter has been used as a measure of voice quality in mammals (Rendall 2003) and in a similar manner to other source-related features, may contribute to vocal distinctiveness (Owren & Linker 1995). In this analysis,

Jitter was calculated by dividing the average absolute difference between consecutive frequencies, by the mean F0 per groan (peak-picking method, Horii 1979; Titze et al. 1987).

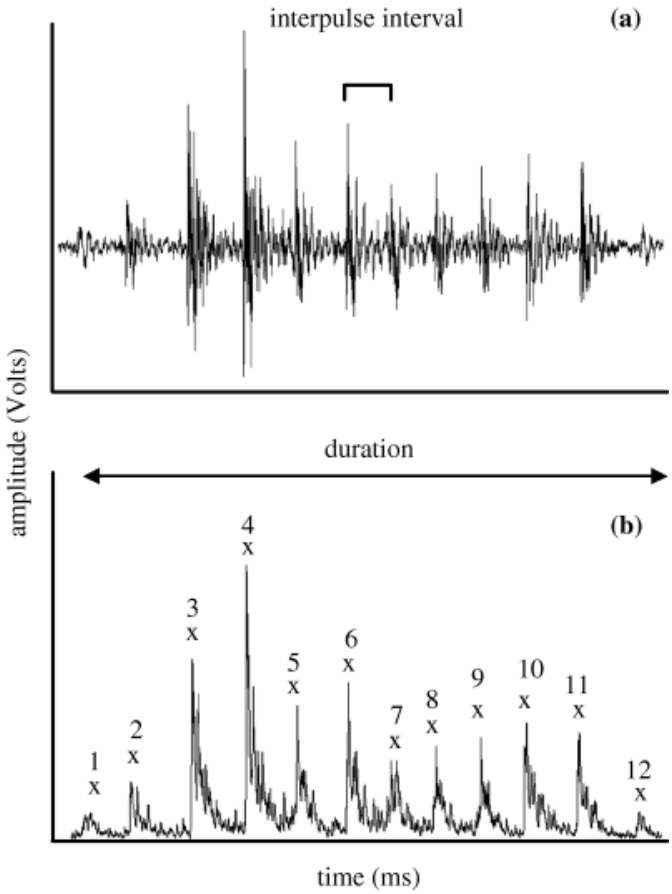

Fig. 2: Waveform of (a) a fallow deer common groan; and (b) its relative envelope. On the envelope of the signal, each amplitude peak is detected and indicated as 'pulse'.

In the spectrograms of common and harsh groans (Fig. 1), pronounced horizontal frequency bands are visible. These bands correspond to peaks in the spectrum and they can result from both the vibration pattern (regular or irregular) of the source and from the vocal tract (i.e. formant frequencies and nasal resonances). The conspicuous frequency bands of fallow deer groans represent formants (McElligott et al. 2006). The values of the first six formant frequencies (F1–F6) were estimated using Linear Predictive Coding analysis (LPC) [Sound: To Formant (burg) command] in Praat 4.3 DSP package. By performing a single LPC analysis on each groan, higher formants (F4–F6) were better detected and therefore more accurately measured than lower formants (F1–F3). We therefore conducted a double or a triple LPC analysis on each groan to get the best estimations of all formants. We first carried out an LPC analysis (time step = 0.01–0.02 s, maximum number of formants = 3–4, maximum formant = 700–850 Hz, window length 0.1–0.26 s) to measure the frequencies of

the first three formants (F1–F3). Then, we performed a second LPC analysis (time step = 0.01–0.02 s, maximum number of formants = 6–7 maximum formant = 1800–2600 Hz, window length 0.1–0.26 s) to estimate the last three formant frequencies (F4–F6). When the sixth formant was not detected by the second LPC analysis (n = 91), we conducted a third LPC analysis (time step = 0.01–0.02 s, maximum number of formants = 5–6 maximum formant = 1800–2600 Hz, window length 0.1–0.26 s). LPC measurements were transferred to Excel. We calculated the minimum frequencies of the six formants ($F1_{min}$–$F6_{min}$) from each groan by averaging the values over the last part of the call when formants become flat (plateau). We included in our analysis the minimum (and not the mean) formant frequencies because they are more likely to be related to individual characteristics (Fitch 1997; Reby & McComb 2003a) and therefore to vary between individuals. We also estimated the minimum spacing of the formants [known as minimum formant dispersion (Df_{min})] according to Reby & McComb (2003a).

To examine the differences in the acoustic structure between common and harsh groans (n = 41 in both cases) we analysed and compared the two types of groans emitted from seven different males (4–8 calls for each individual, average = 6.4 ± 0.75). The same data set was also used to evaluate the intra-individual stability in the spectral structure across groan types. To investigate the individual-related variation in common groans, we constructed a larger data set required by this type of analysis; we analysed 153 groans from 16 different males (7–10 calls for individual, average = 9.6 ± 0.24) ranging from 5 to 8 yr old.

Statistical Analysis

We used the Wilcoxon signed-ranks test to investigate the differences between the acoustic structure of common and harsh groans. We compared the averages of the minimum formant frequencies, the minimum formant dispersion and the maximum vocal tract length between the two types of groans.

We quantified the individual distinctiveness of the common groans by performing a principal component analysis (PCA) followed by a multivariate analysis of variance (MANOVA) and a discriminant function analysis (DFA). PCA and DFA require a full data matrix. Due to the different quality of the recordings, it was not possible with some groans (n = 35) to measure all the acoustic parameters and therefore our data set contained missing values. To preserve the full matrix (n = 153 groans) we substituted missing values with the

average of each variable (Blumstein & Munos 2005). Moreover, we log transformed some of the acoustic variables (pulses, duration, $F0_{mean}$, $F0_{min}$, and $F0_{max}$) to reach normal distributions. We used PCA to eliminate redundancy due to the high intercorrelation of the acoustic variables in our data set and to examine clustering among variables. PCA transforms a large number of correlated variables into a few orthogonal variables (principal components) that explain the maximum amount of variation in the original data set with a minimum loss of information. Principal components are weighted linear combinations of the original variables. We retained those components with eigenvalues greater than 1 (Kaiser's criterion) and applied a Varimax rotation of axes to improve component interpretation. The scores of the five extracted principal components were tested and confirmed for normality (Kolmogorov–Smirnov test) and used as input variables in the DFA.

We used a multivariate analysis of variance (MANOVA) to confirm statistical differences in the principal component scores across individuals. We used DFA to quantify the extent to which individuals can be classified on the basis of their common groans and which group of variables account most for this classification (Johnson & Wichern 1992). DFA identifies a linear combination of values (canonical functions) from two or more discriminating variables (component scores) that best separate cases (groans) into their *a priori* assigned discrete classes (individuals). On the basis of the discriminant functions, each groan was assigned to its appropriate group (correct assignment) or to another group (incorrect assignment). We calculated the percentage of correct classification due to chance by applying a randomization procedure. The expected level of correct assignment was averaged from DFAs performed on 1000 randomized permutations of the data set (Solow 1990; McGarigal et al. 2000). We cross-validated our results by performing a leave-one-out classification, an appropriate method for small sample sizes (Lachenbruch & Mickey 1968; Goutte 1997; McGarigal et al. 2000).

Some principal component scores had similar factor loadings on the same discriminant function. Therefore, we conducted a second DFA to clarify the relative contribution of specific features in discriminating among individuals and to verify whether all the measured acoustic variables are important for individual separation. In this new analysis, we included all of the measured parameters and used a forward stepwise procedure (Johnson & Wichern 1992). Variables were entered in this analysis based on the change in Wilk's lambda (F to enter = 3.84; F to remove = 2.71).

To investigate whether formant-related information about individuality carry over across groan types, we examined if male common and harsh groans, considered as a set, were consistently different between individuals. For this purpose, we first built a sample with common and harsh groans combined as they represented a single call type. Then, we conducted a univariate analysis of variance (ANOVA) on each of the seven variables considered ($F1_{min}$–$F6_{min}$ and Df_{min}) to test for between-male differences. We used Kolmogorov–Smirnov test to determine if the variables were normally distributed. The randomization test was performed using R 2.0.1. (R Development Core Team 2004). All the other statistical analyses were conducted using SPSS 11.5. All tests were two-tailed and factors were considered to have a statistically significant influence if $p < 0.05$. All means are given with standard errors.

RESULTS

Acoustic Characteristics of Common and Harsh Groans

Common groans are characterized by a pulse-train structure (Fig. 1a). Groans lasted from 230 to 607 ms (n = 153; mean = 383.0 ± 6.1 ms) and contained a number of pulses, ranging from 7 to 18 per groan (n = 153; mean = 11.0 ± 0.2). The $F0_{mean}$ was low and varied from 21 to 39 Hz (n = 153; mean = 28.2 ± 0.3 Hz). The $F0_{min}$ ranged from 16 to 29 Hz (n = 153; mean = 22.3 ± 0.2 Hz) while the $F0_{max}$ varied from 22 to 55 Hz (n = 153; mean = 34.7 ± 0.5). The variability of the fundamental frequency along the call was quantified by the jitter parameter, which ranged from 2.0 to 29.6% (n = 153; mean = 12.6 ± 0.4%). There were six formants within the first 2.5 KHz of frequency of the common groans. The first two formants are flat over the whole groan. The $F1_{min}$ ranged from 152 to 263 Hz (n = 134; mean = 208.5 ± 2.0 Hz) whereas the $F2_{min}$ ranged from 329 to 496 Hz (n = 138; mean = 414.3 ± 1.9 Hz). From the third formant upwards, frequencies decrease along the call until they reach a minimum value at the plateau. The minimum frequency ranged from 457 to 677 Hz for F3 (n = 149; mean = 575.2 ± 3.3 Hz), from 966 to 1149 Hz for F4 (n = 153; mean = 1060.2 ± 2.9 Hz), from 1170 to 1371 Hz for F5 (n = 153; mean = 1265.9 ± 3.2 Hz), and from 1672 to 1974 Hz for F6 (n = 147; mean = 1806.9 ± 5.2 Hz). The Df_{min} varied from 281 to 326 Hz (n = 132; mean = 300.6 ± 0.8 Hz).

Harsh groans are typically emitted in a short series, preceded and followed by common groans. They constitute most of the post-copulatory vocalizations (McElligott &

Hayden 2001) and they are also often associated with marking behaviour (E. Vannoni, personal observation). The F0 of harsh groans is not well defined (Fig. 1b) and while two to three pulses are usually visible at the beginning of the groan, the pulse-train structure disappears. The formants are clear and are usually flat or show a weak modulation over the length of the groan. The minimum frequencies of the last four formants ($F3_{min}$–$F6_{min}$) of the harsh groans were significantly different than in common groans (Table 1). However, the minimum formant dispersion did not differ between the two types of groans.

Table 1: Acoustic parameters measured on common and harsh groans (n = 41 for both) from males (n = 7) that provided both types of groans.

	Common groans		Harsh groans			
Acoustic parameter	Mean	SE	Mean	SE	p	z
$F1_{min}$ (Hz)	211.22	3.05	199.36	6.05	0.13	-1.52
$F2_{min}$ (Hz)	412.83	5.80	416.20	4.58	0.40	-0.85
$F3_{min}$ (Hz)	570.67	5.70	514.72	5.57	0.02	-2.37
$F4_{min}$ (Hz)	1055.03	10.27	1079.42	12.58	0.03	-2.20
$F5_{min}$ (Hz)	1253.37	9.53	1213.98	11.84	0.03	-2.20
$F6_{min}$ (Hz)	1791.09	24.82	1851.82	21.59	0.03	-2.20
Df_{min} (Hz)	298.49	3.03	302.58	2.54	0.24	-1.19
VTL_{max} (cm)	58.67	0.59	57.86	0.48	0.24	-1.19

Acoustic Cues to Identity

Five components (PC1–PC5) that exceeded Kaiser's criterion (eigenvalues greater than 1) were generated from the PCA performed on the common groans (n = 16, Table 2). These five components combined accounted for 82% of the variation in the original data set. Filter-related parameters were grouped in two different components (PC1 and PC4) that reflect different aspects of the vocal tract function. PC1 included higher formant frequencies ($F3_{min}$–$F6_{min}$), which are mainly related to the length of the vocal tract, whereas PC4 consisted of lower formants ($F1_{min}$–$F3_{min}$) generally associated to the shape of the vocal tract. Two components reflected aspects of source characteristics; PC2 represented measures of the F0 contour ($lgF0_{mean}$, $lgF0_{max}$ and $lgF0_{min}$), whereas PC5 included $lgF0_{min}$, $lgF0_{max}$ and the Jitter, a measure of voice quality based on the stability of vocal folds vibration. Finally, temporal parameters (lgPulses and lgDuration) were highly correlated with PC3. Groans were significantly different among males for all principal component scores (MANOVA: PC1,

$F_{15,137}$ = 10.47; PC2, $F_{15,137}$ = 14.76; PC3, $F_{15,137}$ = 2.90; PC4, $F_{15,137}$ = 5.14; PC5, $F_{15,137}$ = 6.04; $p < 0.001$ in all cases).

Table 2: Rotated factor loadings of the measured acoustic variables on the five PCs with eigenvalues >1.

	Components				
Acoustic variable	1	2	3	4	5
Df_{min}	**0.95**	0.03	-0.04	0.17	0.06
$F6_{min}$	**0.91**	0.04	-0.06	0.00	0.05
$F5_{min}$	**0.88**	0.01	-0.01	0.15	-0.05
$F4_{min}$	**0.86**	0.04	0.09	-0.19	0.02
$lgF0_{mean}$	0.01	**0.96**	-0.01	-0.02	0.09
$lgF0_{max}$	0.01	**0.83**	0.08	0.02	**0.44**
$lgF0_{min}$	0.12	**0.78**	-0.10	-0.05	**-0.49**
lgPulses	-0.00	0.29	**0.95**	-0.02	0.00
lgDuration	-0.00	-0.31	**0.94**	-0.01	0.01
$F1_{min}$	-0.33	-0.08	0.07	**0.77**	0.04
$F3_{min}$	0.28	-0.19	-0.13	**0.67**	-0.23
$F2_{min}$	0.17	0.20	0.01	**0.57**	0.21
Jitter	0.07	0.10	-0.01	0.93	**0.92**
Eigenvalue	3.48	2.49	1.82	1.46	1.41
% variance explained	26.77	19.16	14.03	11.21	10.81

Cross-validated DFA classified 36.6% of the male common groans correctly. The mean percentage of correct assignment due to chance calculated by the randomization test was 6.19 ± 0%. Because none of the 1000 data sets had a correct classification greater than or equal to the original data set, groans were correctly classified at a percentage higher than that expected by chance at $p < 0.001$. Five significant discriminant functions were generated (Ps < 0.01, Table 3). The first two discriminant functions together accounted for 81.1% of the variation and were correlated with scores from the PC1 and PC2. The third discriminant function accounted for another 10.6% and was correlated with scores from PC3, PC4 and PC5. The remaining two discriminant functions were residual in nature, but combined explained 8.3% of the variance.

Table 3: Structure matrix from the DFA, conducted using the five PC scores. The matrix shows the correlations between the PC scores and the discriminant functions and therefore the relative contribution of each variable to the separation of individuals.

	Functions				
PC scores	1	2	3	4	5
PC1	**0.47**	**0.45**	-0.07	**-0.63**	-0.42
PC2	**0.60**	**-0.50**	-0.26	0.37	0.42
PC3	0.03	0.06	**0.46**	-0.41	**0.79**
PC4	0.14	0.32	**0.51**	**0.78**	-0.05
PC5	-0.05	0.41	**-0.66**	0.24	**0.57**
Eigenvalue	2.95	1.75	0.61	0.26	0.22
% variance explained	50.90	30.20	10.60	4.50	3.80

Not all the acoustic variables significantly contributed to the assignment of common groans to the correct individuals, when they were used in the stepwise DFA. Seven variables entered the analysis: four formant frequencies ($F2_{min}$, $F3_{min}$, $F5_{min}$ and $F6_{min}$), two F0-related parameters ($lgF0_{min}$ and $lgF0_{mean}$) and one temporal parameter (lgDuration). The $lgF0_{min}$ entered the analysis first followed by the $F6_{min}$ and the $lgF0_{mean}$. The cross-validated percentage of correct assignment was of 53.6%.

When common and harsh groans were considered as a single call type, the univariate analyses of variance indicated significant heterogeneity among individuals for most of the formant frequencies and for the minimum formant dispersion (ANOVAs: $F1_{min}$, $F_{6,75} = 1.69$, $p = 0.14$; $F2_{min}$, $F_{6,75} = 5.75$, $p < 0.01$; $F3_{min}$, $F_{6,75} = 0.22$, $p = 0.97$; $F4_{min}$, $F_{6,75} = 6.69$, $p < 0.01$; $F5_{min}$, $F_{6,75} = 2.57$, $p < 0.05$; $F6_{min}$, $F_{6,75} = 11.53$, $p < 0.01$; Df_{min}, $F_{6,75} = 7.78$, $p < 0.01$).

DISCUSSION

We found that the fundamental frequency-related parameters and minimum frequencies of the highest formants accounted for >80% of the variation, in the individually distinctive common groans of fallow bucks. It was possible to correctly classify the groans of 16 individuals in 36.6% of cases, and in 53.6% of cases using the stepwise procedure. Minimum formant frequencies still showed significant inter-individual variation when common and harsh groans were combined, indicating that some information about individuality holds across groan

types. Studies investigating vocal individuality in mammals such as humans and other primates, have shown the prominent role of vocal tract resonances as cues to individual identity (Owren et al. 1997; Bachorowski & Owren 1999). Our results suggest that in fallow deer, source and filter-related features contribute equally to the vocal identity, possibly because of different selection pressures acting on the acoustic structure of groans.

Common groans were characterized by a series of low-frequency glottal pulses produced by the larynx. Pulses contain broadband energy and therefore emphasize the resonances of the vocal tract (Fitch 1997; Riede & Zuberbühler 2003). In fallow deer, these resonances are likely to convey important information such as the body size of the caller (Fitch & Reby 2001; McElligott et al. 2006). The production of pulse-train vocalizations may therefore have evolved to broadcast formant-related information in a more efficient way. However, formant frequencies play a crucial role in the vocal communication of red deer (*Cervus elaphus*), in which males mainly produce vocalizations characterized by a harmonic structure, sometimes interrupted by non-linear phenomena, and with higher F0 than that of groans (Reby & McComb 2003a; Reby et al. 2005). The extremely low F0 and the pulse structure of fallow deer groans suggest a similar production mechanism to that of the human pulsed phonation (Blomgren et al. 1998). This phonation mode is characterized by reduced subglottal pressure (associated with the expiratory force) and lower airflow than other phonation types (Blomgren et al. 1998). Pulse signals are therefore physiologically easy to produce (Riede & Zuberbühler 2003) and may require less effort from the vocalizing individual. Fallow bucks commonly groan at rates of 60 per minute and can also groan at rates of 90 for very short periods (McElligott & Hayden 1999, 2001). Such high vocalization rates not only play a role in intrasexual competition, but are also probably necessary to facilitate the detection and the recognition of the information contained in the vocalizations in a situation where many conspecifics vocalize in close proximity (Jouventin et al. 1999; McElligott & Hayden 1999). We suggest that the production of pulsed vocalizations could be adaptive if this would allow males to save energy and therefore to achieve and sustain high vocalization rates. However, data on the aerodynamics and physiological properties of the vocal production mechanism in fallow deer are needed to test this hypothesis.

Formants showed a weak modulation along the harsh groans in a similar manner to red deer harsh roars. This suggests a static posture of the vocalizing male and the onset of the vocalization when the larynx is already pulled down towards the sternum (Reby & McComb

2003b; McElligott et al. 2006; this study). The observed differences in formant values between common and harsh groans may represent differences in the vocalization posture or articulatory movements (movement of the tongue, mandible, and lips) during the production of the two types of groans (Rendall et al. 1998). However, there was no significant difference in the minimum formant dispersion between the two types of groans, suggesting a similar extent of laryngeal lowering.

Discriminant function analyses confirmed that fallow deer common groans are individually distinctive. The percentage of correct assignment (36.6%) was significantly higher than that expected by chance (6.3%), but lower than that previously reported for fallow deer groans (87.9% using network classification; Reby et al. 1998). This discrepancy is probably due to the larger number of individuals used in our analysis and for statistical reasons, this results in a lower classification rate. Moreover, it is important to consider that our result represents a conservative estimate of the percentage of correct assignment. The loss of about 20% of the variance in the acoustic properties due to the use of the PCA probably reduced the number of groans that were assigned to the correct individuals. This hypothesis is supported by the higher percentage of correct assignment that we obtained from the stepwise DFA (53.6%), in which the acoustic parameters (and not the principal component scores) were used, and all available information was therefore preserved. Generally, with mammal vocalizations, the variety of acoustic parameters included in DFA and the diverse sample sizes used to examine the degree of individuality, make it difficult to compare the absolute values of correct assignment across studies. Nevertheless, we found that calls were assigned to the correct individual with a probability of 6 (DFA with PC scores) to 8.5 (stepwise DFA) times higher than that predicted by chance, a result similar to that reported for other mammals such as baboons (Owren et al. 1997; Fischer et al. 2002).

The examination of the structure matrix of the DFA (Table 3) indicates that characteristics associated with source energy, vocal tract filter, and temporal features, all contribute to the identification of a given common groan, and with variables related to the F0 contour and the minimum frequencies of the higher formants being the most distinctive. This finding is consistent with those on acoustic individuality in African elephants (*Loxodonta africana*), baboons (*Papio hamadrayas ursinus*), and humans showing that both the vocal folds and supra-laryngeal structures play important roles in shaping the call and producing inter-individual acoustic differences (Bachorowski & Owren 1999; Rendall 2003; Soltis et al.

2005). However, we found that variation in the vocal-tract resonances did not provide more stable, individually distinctive acoustic information than variation in vocal fold activity. Instead, the similar values of the correlations between the scores of PC1 and PC2 and the first two discriminant functions (Table 3) indicate a comparable contribution of filter-related characteristics (especially those related to the vocal tract length) and source-related characteristics in differentiating individuals, with the latter being slightly more important. Nonetheless, filter-related features that were not measured in this study, such as formant amplitudes and spectral shape, could also contribute to vocal distinctiveness (Rendall 2003) and lead to a larger influence of vocal tract resonances on acoustic individuality.

Results from the stepwise DFA indicated that the discrimination power of the component in which variables of the F0 contour were included, is mainly determined by the minimum F0 ($F0_{min}$), followed by the mean F0 ($F0_{mean}$). $F0_{min}$ represents the minimum rate at which the vocal folds vibrate and this is physiologically constrained by their length (Titze 1994; Fitch 1997). It is therefore not surprising that the $F0_{min}$ is the acoustic feature that varies most among individuals. Pulsed vocalizations generally show a narrow range of variation of F0 (Blomgren et al. 1998; Riede & Zuberbühler 2003; Frey et al. 2006). This suggests a highly stabilized vibration pattern, which is likely to contribute to the stability of the F0-related parameters within individuals. However, sexual selection might have favoured the evolution of vocal cues to the fitness-related characteristics in fallow deer males, and led to the selection of lower pitch vocalizations, indicating higher-quality individuals. Such low pitch may persist and be perceived at distances from the caller at which formants are likely to be distorted (O'Connell-Rodwell et al. 2000; McComb et al. 2003).

Although all filter-related characteristics potentially represent cues to caller identity, lower formants contribute less than higher ones to the inter-individual variability of the acoustic structure of common groans. One explanation based on what is known about human vocal production is that while lower formants are determined by changes in the shape of the vocal tract occurring continuously during vocalization, higher formants may be less variable because they represent the resonance effects that are primarily determined by the length of the vocal tract (Fant 1960; Reby & McComb 2003a). However, the extent to which the vocal production mechanism in fallow deer (especially in terms of vocal tract shape and vocal folds dynamics) is similar to that of humans remains to be investigated. In our study, the frequency of the highest formant ($F6_{min}$) entered the stepwise analysis first and this was the variable that

most strongly related to the minimum formant dispersion (Df_{min}). Formant dispersion is related to vocal tract length and in turn to the body size of a variety of mammals (Fitch 1997; Riede & Fitch 1999; Reby & McComb 2003a). Therefore, individual variation in formants might be traced to some extent to differences in body size between individuals.

Other factors related to the source energy (Jitter) and temporal pattern are likely to play a role in individual vocal distinctiveness in fallow deer common groans, because the scores of their component (PC3 and PC5, respectively) are correlated with the third discriminant function (Table 3). Results of the stepwise DFA showed that the duration of a groan explained a small percentage of inter-individual variation in the acoustic structure and contributed to improved classification accuracy. In contrast, Jitter did not enter the analysis and therefore appears to have a marginal (if any) role in determining vocal individuality. However, because of the variety of methods that can be used to calculate Jitter (reviewed in Titze & Liang 1993), these results are only applicable to our method of measuring Jitter. Although Jitter may not differ substantially between individuals, it represents a measure of vocal perturbation and may therefore provide information about arousal states (Gamba & Giacoma 2005; Riede et al. 2005).

Individual characteristics of vocalizations can be specific to each call type or can be shared by several call types. The acoustic structure of the harsh groans mainly consists of broadband energy determined by irregular vibrations of the vocal folds. This non-periodic energy source generates noise that in the spectrum looks like deterministic chaos (Wilden et al. 1998). While the presence of this non-linear phenomenon in calls of certain individuals (and not of others) may provide an additional cue to individuality (Fitch et al. 2002), it makes all the information-related to the F0 unavailable to receivers. Formants have been hypothesized to represent a highly stable and reliable source of information about the identity of the caller carrying this information across a variety of vocalization contexts and call types (Owren et al. 1997). Results from our univariate analysis of variance performed on a combined set of harsh and common groans indicated that the vocal tract resonances of the two types of groans share cues to identity. Fallow deer might therefore possess individual voices as shown in other mammals (humans, Doddington 1985; rhesus monkeys, *Macaca mulatto*, Rendall et al. 1998, red deer, Reby et al. 2006). However, it is unlikely that these cues alone allow individual recognition because of the large contribution to individuality of the source-related characteristics.

Bouts of harsh groans are more likely to be delivered when other males are close to the vocalizing individual (E. Vannoni, personal observation) and therefore when other sensory channels (such as vision or olfaction) are available for recognition. In this context, it could be more beneficial for males to produce vocalizations with non-periodic energy sources, such as harsh groans, in which the information about individuality is reduced (Rendall et al. 1998) but other formant-related information such as body size is emphasized (Fitch et al. 2002; Reby et al. 2005). Morton (1977) suggested that harshness is tied to large body size indirectly through the production of low-pitched sounds. However, in homoeothermic vertebrates, size at maturity is constant and therefore the use of low or high frequency sounds might be more important in conveying information about motivation, than about body size (Morton 1982). In mammals, although harshness is generally associated with high subglottal pressure and therefore likely to reflect the motivational state of the caller (Reby & McComb 2003b), it is also characterized by broadband energy that enhances the transmission of body size information related to formants (Fitch et al. 2002; Reby et al. 2005).

The phonic structure of fallow buck groans differs greatly from the naturally selected contact calls of adult female fallow deer. Adult females mainly produce tonal calls with relatively high fundamental frequency, and calls composed of a first noisy part and a second part with harmonic structure (Torriani et al. 2006). Some female calls are characterized by a low pitch and a noisy structure. In these, a small decrease in formants is evident along the call, indicating a small vocal tract elongation due to the lowering of the larynx (McElligott et al. 2006; Torriani et al. 2006). The fundamental and formant frequencies of female calls are considerably higher than those of male groans. This is not surprising given the sexual size dimorphism (McElligott et al. 2001), and therefore females have shorter vocal folds and vocal tract. Moreover, the sterno-thyroid and sterno-hyoid muscles, which allow the descent of the larynx towards the sternum, are highly sexually dimorphic (Reby & McComb 2003b).

To conclude, this is the first time that the individually distinct acoustic structure of fallow deer common groans was examined from a source-filter theory perspective. Our results suggest that formant frequencies produced by the filtering process in the vocal tract and acoustic characteristics related to voice source, play similar roles in coding for individuality. This study also provides the first quantitative description of the acoustic structure of harsh groans, and suggests that this structure carries some of the information about individuality. Whether the variation in the acoustic cues to individuality are used by the animals for

individual recognition remains to be investigated. However, given that fallow deer fawns can identify their mothers based on their individually distinctive vocalizations (Torriani et al. 2006), then it is also likely that adults can identify individuals when acoustic cues to individuality are available in calls.

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CHAPTER 5

Fundamental frequency and formant frequencies of groans encode information about male quality in fallow deer (*Dama dama*)

Submitted

Fundamental frequency and formant frequencies of groans encode information about male quality in fallow deer (*Dama dama*)

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ABSTRACT

Models of honest advertisement predict that sexually selected calls should signal male quality. In most vertebrates, high quality males have larger body sizes that determine higher social status and in turn higher reproductive success. Previous research has emphasized the importance of the formant frequencies of calls as cues to body size in mammals. However, the role of the acoustic features of the vocalizations as cues to other quality-related phenotypic characteristics of callers has been rarely investigated. We investigated whether the acoustic structure of fallow deer groans provide reliable information on the quality of the caller, by examining the relationships between the frequency components of calls (fundamental frequency and formant frequencies) and male quality (body size, dominance rank, and mating success). We found that fundamental frequency-related parameters were not related to body size. However, larger males produced groans with lower formant frequencies. Groans of high-ranking males were characterized by lower minimum fundamental frequencies and to a lesser extent, by lower formant dispersions. Dominance rank was the factor most strongly related to mating success, with higher-ranking males having higher mating success. The minimum fundamental frequency and the minimum formant dispersion were only indirectly related to male mating success (through dominance rank). Our study reveals that the acoustic components of sexually selected fallow deer groans can provide accurate information on the quality of the caller. This information is likely to be used by males for mutual assessment during agonistic interactions and as a cue for mate choice by females.

INTRODUCTION

Male vocalisations are often subject to sexual selection and can be used to assess the quality and condition of the caller in various vertebrates (Andersson 1994; Doty & Welch 2001; Christie et al. 2004; Fischer et al. 2004). The reliability of the information encoded in sexually selected acoustic signals is maintained by constraints imposed on the caller which make cheating costly (Vehrencamp 2000). Vocalisations that are physiologically or physically constrained carry some characteristics that are directly related to intrinsic properties of the caller and therefore cannot be faked (Maynard-Smith & Harper 2003).

The relationships between the body size of callers and the acoustic parameters of vocalisations are of particular interest in species where body size determines fighting ability and reproductive success (McElligott et al. 2001; Reby & McComb 2003; Price et al. 2006; Pfefferle & Fischer 2006). In toads, frogs, and birds, body size is negatively related to the fundamental frequency of calls (Davies & Halliday 1978; Ryan & Brenowitz 1985; Bee et al. 1999). In mammals, and within a given species, body size is related to fundamental frequency across age categories and among adult females but not among adult males (Fischer et al. 2002; Collins and Missing 2003; Reby & McComb 2003; Pfefferle & Fischer 2006). Fundamental frequency is determined by different factors such as the length of the vocal folds, longitudinal stress on the vocal folds, and the tissue density of the vocal folds (Titze 1994). For example, longer vocal folds result in lower fundamental frequency. Because the larynx is not constrained by the bones of the skull, the vocal folds may grow independently of the rest of the head or body (Fitch 1997). Moreover, vocal folds are highly sensitive to changes in testosterone (Beckford et al. 1985; Dabbs & Mallinger1999) and they may grow longer in males with higher testosterone. Thus, fundamental frequency is a poor indicator of male body size in mammals.

In mammals, formant frequencies or resonances of the vocal tract probably represent the key acoustic variables linked to variation in body size (Fitch 1997; Fitch & Giedd 1999; Riede & Fitch 1999; Reby & McComb 2003; Harris et al. 2006). Formants and their average spacing (formant dispersion) depend upon tissue structure, and the shape and length of the vocal tract (Fant 1960). The longer the vocal tract, the lower the formants frequencies became. In contrast to the larynx, the vocal tract is fixed to the skull, and therefore closely tied to overall body size (Fitch 1997).

In addition to male body size, sexually selected calls might convey information about other indirect measures of male fitness. Social dominance rank and acoustic parameters are both phenotypic characteristics of the caller and they can be indirectly related if both are influenced by other caller characteristics, such as the physiological state (Beckford et al. 1985; Mazur & Booth 1998; Beehner et al. 2006). The perception of these characteristics based on acoustic cues by competing males may affect the outcome of agonistic interactions. Females evaluating the relative quality of males might rely on acoustic cues related to dominance since high-ranking males often have better survival, and overall reproductive success than low-ranking males (McElligott et al. 2001, 2002; Alberts et al. 2006). While there is now strong evidence that some acoustic parameters of vocalisations represent body size, the relationships between vocal parameters and other characteristics linked to male quality, such as dominance rank and mating success, have rarely been investigated.

Fallow deer are ideal for investigating the role of acoustic signals as indicators of male quality. Fallow deer are characterised by a polygynous mating system with high male-male competition and skewed reproductive success (McElligott et al. 1998; McElligott & Hayden 2000). Larger males are generally higher ranked than smaller males, and rank is also closely associated with mating success (McElligott et al. 2001). Males only vocalise during the breeding season and the sexually selected call they produce is known as a groan (Vannoni & McElligott 2007; Reby et al. 1998). In the northern hemisphere, males start groaning approximately three weeks before the first matings take place (late September) and continue until the vast majority of matings have occurred (early November, McElligott et al. 1999). Vocalisations are directed both towards males during agonistic encounters and towards females during chasing or herding behaviour, suggesting a potential role of groans in both male-male competition and female choice (McElligott & Hayden 1999). When males vocalise, the larynx is pulled down towards the sternum and the length of the vocal tract increase. As a consequence, the formant frequencies decrease and reach a minimum value that could potentially reveal information related to male quality (Reby & McComb 2003; McElligott et al. 2006). The groans of fallow bucks are individually distinctive (Vannoni & McElligott 2007; Reby et al. 1998) and as is the case with red deer (*Cervus elaphus*), conspecifics are likely to recognise males based on the sound of their calls (Reby et al. 2001).

We investigated the relationships between the acoustic structure of fallow deer groans and male quality. We first determined whether body size is related to the fundamental

frequency-related parameters, formant frequencies, and formant dispersion of groans. We then examined the relationships between some of these acoustic parameters, and dominance rank and mating success, while also considering the role of body size.

METHODS

Study site and population

The study was conducted on a herd of European fallow deer in Phoenix Park (709 ha, 80% pasture, 20% woodland; 53° 22' N, 6° 21' W), Dublin, Ireland. All males used in this study were of known age, tagged and were therefore individually recognisable.

Morphological measurements

We used measurements taken from 17 different males (5 in 1996, 1 in 1997, 1 in 1999, 3 in 2001, 6 in 2002 and 1 in 2003). Males were caught immediately before (third week of September) or after the breeding season (third week of November). The males were immobilised by a veterinary surgeon using gas-propelled darts and then measured. The measurement of a segment of one hind leg for each male was used as a proxy indicator of skeletal size (Clutton-Brock et al. 1988; Coltman et al. 1999). The males in this study did not suffer any long-lasting effects as a result of being handled. Full details of the immobilising, catching and measuring protocols are contained in McElligott et al. 2001.

Observations

We conducted behavioural observations during the rut in 1997, 2000, 2002 and 2003. The rut refers to the period during which matings occur. During this time, an all-event recording of agonistic interactions and matings was carried out every day from dawn to dusk (circa 11 hours per day). There were 7-13 observers in the field at all times and the coverage of animals was maximised. The measure of male mating success was based on the number of observed copulations, and this provides a very good estimator of their reproductive success (Say et al. 2003).

Dominance relationships

The outcomes of the agonistic interactions were used to calculate the dominance rank of each male by applying the David's score method (David 1987). This method is the most appropriate when interactions are recorded over a long period of time, because it takes into

account repeated interactions between dyad members that may determine win /loss asymmetries (Gammell et al. 2003). Dominance ranks were calculated for males that interacted with at least 10% of other mature males.

Recording and selection of groans

Recordings were made using a Sennheiser MKH 70 directional microphone connected to a Sony digital audio tape recorder DAT-TCD D100. Groans were recorded between dawn and sunset at a distance of 10 to 50 m from the vocalising animal.

Vocalisations were imported into a computer using Avisoft-SASLab Pro 4.38 at a sampling rate of 22.05 KHz and saved in WAV format, and at 16-bit amplitude resolution (Vannoni et al. 2005). The recordings that did not contain energy above 8 KHz were down-sampled to 16 KHz for a better frequency resolution. Narrow-band spectrograms of groans (Fig. 1a, FFT method, window length = 0.03 s, time step = 1000, frequency step = 250, frequency resolution = 20 Hz, Gaussian window shape, dynamic range = 35 dB) were edited using Praat 4.5.01 DSP package (P. Boersma & D. Weenink, University of Amsterdam, The Netherlands). Vocalisations with high levels of background noise were not considered for analysis.

During the breeding season, fallow deer males feed very little and lose approximately 26% of their body weight (McElligott et al. 2003), and there is some evidence from primates and deer that the acoustic structure of vocalisations can be affected by exhaustion (McComb 1988; Fischer et al. 2004). We therefore analysed recordings taken between October 8 and October 20 when only a small proportion (15% or less) of the total number of matings had usually occurred (McElligott et al. 2001), and the majority of agonistic interactions among males were non-contact displacements (McElligott et al. 1998). This minimised the possibility that variation in the phonic structure of groans could have occurred due to exhaustion of the animals.

For each male, we selected groans from different bouts that were recorded during one or more days. We included in our analysis males between 5 and 8 years of age because they had reached their asymptotic size and were not undergoing changes associated with senescence. Moreover, this range of ages includes the males that account for the vast majority of matings (McElligott et al. 2002).

Acoustic analysis

Groans are low-pitched vocalisations and are generally characterised by a pulse-train structure (Fig. 1a). The pulses represent the vibrations of the vocal folds and determine the fundamental frequency (F0) of the call. Fundamental frequency is defined as the inverse of interpulse interval and this can be measured as the distance between consecutive pulse onsets (Fig. 1b). Distances between pulses were measured automatically from the envelope (amplitude vs. time) of the signal by using Pulse Train Analysis (Fig. 1b; method = rectification + exponential decay; pulse detection = peak search with hysteresis; time constant = 1 ms; threshold = 0.1 V; hysteresis = 16-19 dB) in Avisoft-SASLab Pro 4.38. We calculated the values of the F0 along the groan and then averaged these values to obtain the mean F0 (F0mean). Because all groans showed at least a modest frequency inflection, the minimum and the maximum F0 (F0min and F0max) were also included in the analysis.

In the spectrogram of groans, six formants are evident as horizontal frequency bands (F1-F6 in Fig. 1a). The decreasing of these formant frequencies along the groan reflects the elongation of the vocal tract occurring during vocalisation (McElligott et al. 2006). Formants were estimated using Linear Predictive Coding analysis (LPC), (Sound: To Formant (burg) command) in Praat 4.5.01 DSP package. By performing a single LPC analysis on each groan, higher formants (F4-F6) were better detected and therefore more accurately measured than lower formants (F1-F3). We therefore conducted a double or a triple LPC analysis on each groan in order to get the best estimations of all formants. We first carried out an LPC analysis (time step = 0.01-0.02 s, maximum number of formants = 3-4, maximum formant = 700-850 Hz, window length 0.07-0.26 s) to measure the frequencies of the first three formants (F1-F3). Then we performed a second LPC analysis (time step = 0.01-0.02 s, maximum number of formants = 6-7 maximum formant = 1800-2600 Hz, window length 0.07-0.26 s) to estimate the last three formant frequencies (F4-F6). When the sixth formant was not detected by the second LPC analysis, we conducted a third LPC analysis (time step = 0.01-0.02 s, maximum number of formants = 5-6 maximum formant = 1800-2600 Hz, window length 0.07-0.26 s). We calculated the minimum frequencies of the six formants (F1min-F6min) from each groan by averaging the values over the last part of the call when formants become flat. This is the time when the larynx is pulled down at the maximum extent. Finally, we also estimated the minimum spacing of the formants (known as minimum formant dispersion, (Dfmin), according to Reby & McComb (2003).

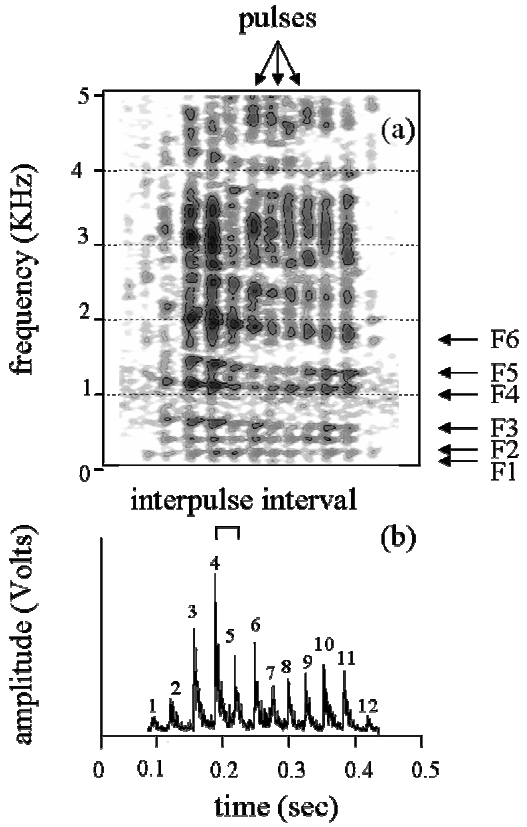

Fig. 1. Narrow band spectrogram of a common groan (a) and its relative envelope (b). On the spectrogram, the pulses and the first six formants are indicated. On the envelope of the signal, each peak of frequency is detected and indicated as "pulse".

We analysed vocalisations of 17 different males recorded during five breeding season between 1997 and 2004 (6 in 1997, 3 in 2000, 2 in 2002, 4 in 2003, and 2 in 2004). Vocalisation recordings and behavioral observations for males were always carried out within the same year. For three males, vocalisations were recorded and body size measurements taken during the same breeding season. For most of the males (N=14), recordings were performed one (N=12) or two years (N=2) before or after their body size measurements were taken. We assumed that the body size measurement that we used (hind leg length) did not change in fully grown mature males.

The low fundamental frequency that characterises fallow deer groans is still detectable and measurable on the spectrogram of groans recorded at certain distances from the source (Vannoni, personal observation). By contrast, formant frequencies are frequently lost or distorted in recordings taken in suboptimal conditions, such as when the microphone is far from the vocalising animal or when it is not facing the microphone (see Vannoni et al. 2005). Therefore, because of the variety of the recording conditions, it was not always possible to

measure the fundamental frequency and the formants on the same groans and for all males. As a result, sample sizes (number of groans and number of males) varied among analyses.

Statistical analysis

We used a general linear mixed effect model (GLMM) procedure fitted with residual maximum likelihood estimation (REML, lme function; Venables & Ripley 2002) to investigate the effect of body size on the acoustic parameters of groans (F0-related parameters: 186 groans; 10.9 ± 1.1 per individual; Formant frequencies and Dfmin: 144 groans; 8.5 ± 0.8 per individual; N = 17). We conducted a univariate GLMM for all the acoustic parameters. Individual identity was fitted as random term so that we controlled for repeated measurements of the same individual. Body size was fitted as a fixed effect.

We used a model selection procedure (Burnham & Anderson 2002) to examine the relationships between the acoustic parameters and other important biological factors, on dominance rank and mating success of the males. In observational studies, such as ours, model selection technique should be preferred to traditional testing approaches (Johnson & Omland 2004; Burnham & Anderson 2002). This technique identifies the model which best describes the structure in a data set among all a priori fitted models considered, controlling for the number of parameters, K, included in each model. Because each model is associated with a biological hypothesis, model selection identifies the hypothesis that is best supported by the data.

Because the sample size we used does not allow us to fit a large number of complex models, we limited our analyses to the factors that we considered most important. Two acoustic parameters of particular biological interest were selected and included in the models. The minimum fundamental frequency (F0min) has the highest degree of inter-individual variation among the acoustic parameters used to describe the phonic structure of fallow deer groans (Vannoni & McElligott 2007). It represents the lowest rate of vocal folds vibration and among the F0-related parameters is the only one to be physiologically constrained (Titze 1994; Fitch 1997). The minimum formant dispersion (Dfmin) is constrained by the length of the vocal tract and is used by red deer males to assess competitors (Fitch 1997; Reby et al. 2005). Moreover, both F0min and Dfmin are related to the reproductive success in red deer (Reby & McComb 2003).

We first formulated two sets of candidate models. The fitted models for dominance rank (First set of models: model 1 to 5, Table 1) included the effects of body size, minimum fundamental frequency (F0min), and minimum formant dispersion (Dfmin). The fitted models for mating success (Second set of models: model 6 to 13, Table 1) included the effects of body size, dominance rank, F0min, and Dfmin. Then, we applied the model selection procedure based on Akaike Information Criterion adjusted for small sample sizes (AICc), (Burnham & Anderson 2002) to each of the two sets. The value of AICc for a given model is a measure of the loss of information resulting from the use of the model to explain a particular pattern. Therefore, the model with the smallest AICc value is estimated to fit best the data set relative to other models considered (Burnham & Anderson 2002). When the difference between the AICc values of two models (ΔAICc) is less than 2 units, both models have support and can be considered competitive. Models with ΔAICc ranging from 3 to 7 have considerably less support by the data, whereas models with ΔAICc > 10 are poorly supported and therefore very unlikely (Burnham & Anderson 2002). Akaike weights (w_i) represent a measure of the strength of evidence for each model and indicate the probability that a particular model is best supported by the data among the other models in the set (Burnham & Anderson 2002). For each model we also calculated the evidence ratio defined as the ratio between the Akaike weight of the best model and the Akaike weights of the competing model. We used the likelihood-ratio tests (LRT) to compare nested models and to assess statistical significance of the factors. The LRT statistics follows a χ^2-distribution with degrees of freedom equal to the difference in the number of parameters.

Data on dominance ranks and mating success were not available for three of the males for which we had measurements of body size. Therefore, to investigate the relationships between acoustic parameters and dominance rank and mating success, we used data from 14 males (F0-related parameters: 156 groans, 11.1±1.3 per individual; Formant frequencies and Dfmin: 115 groans, 8.2±0.9 per individual). We log-transformed dominance rank and mating success to achieve normality. One unit was added to the mating success of all individuals before applying the log-transformation. In this way, we were able to transform the value of those individuals who did not get any matings (value = 0). All analyses were performed in R for Windows version 2.0.1. All tests were 2-tailed and factors were considered to have a statistically significant influence if $p < 0.05$. All means are given with standard errors.

RESULTS

Relationships between body size and the acoustic parameters

The segment of the hind leg that we used as indicator of male body size, ranged from 30.0 to 33.5 cm (mean = 32.1 ± 0.3). Fundamental frequency-related parameters were not significantly related to body size (GLMM: F0min, $F_{1,15}$ = 0.0002, P = 0.990, F0mean, $F_{1,15}$ = 0.0462, P = 0.833, F0max, $F_{1,15}$ = 0.0520, P = 0.823). The first four minimum formant frequencies tended to decrease with increasing body size, but none of the relationships were significant (GLMM: F1min, $F_{1,15}$ = 1.16, P = 0.298; F2min, $F_{1,15}$ = 3.21, P = 0.094; F3min, $F_{1,15}$ = 2.08, P = 0.171; F4min, $F_{1,15}$ = 3.13, P = 0.097). There was a tendency for the minimum frequency of the fifth formant to be negatively related to body size (GLMM: F5min, $F_{1,15}$ = 3.92, P = 0.066). The minimum frequency of the sixth formant and the formant dispersion varied negatively with body size (GLMM: F6min, $F_{1,15}$ = 4.58, P = 0.049; Dfmin, $F_{1,15}$ = 18.93, P < 0.001; Fig. 2).

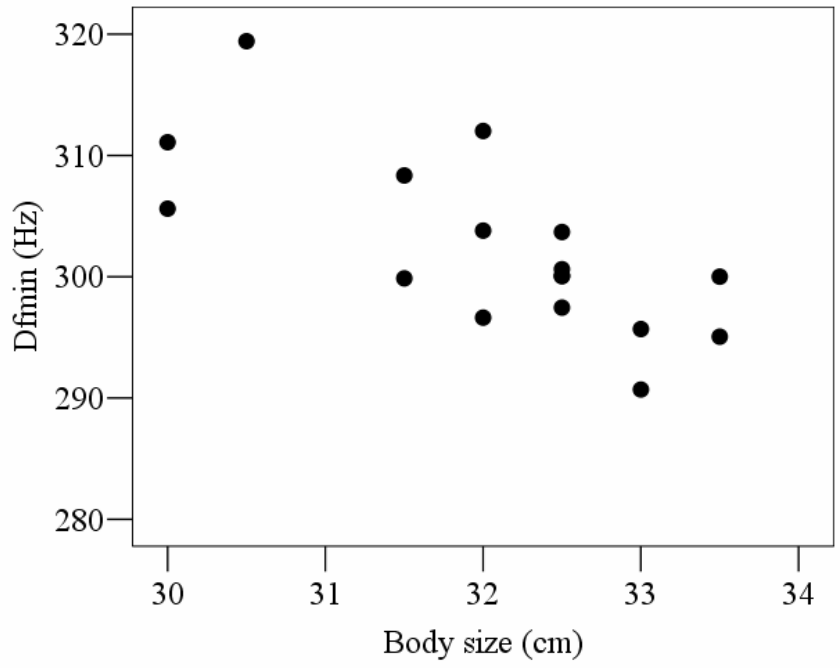

Fig. 2. The relationships between body size and minimum formant dispersion (Dfmin), (n = 17 males, n = 144 groans).

Relationships between acoustic parameters and dominance rank

Model selection favoured the model incorporating the minimum fundamental frequency (F0min), (lowest AICc; Table 1, model 2). The selected model shows that groans characterised by lower F0min are produced by higher-ranking males (model 2; Fig. 3). A close competitor of model 2 was model 4 which only included the minimum formant dispersion (Dfmin), (Table 1, Model 2 and 4: ΔAICc < 2). The two models with the lowest AICc (model 2, 4) were together 64.8% supported by the data (combined Akaike weights, 0.443 and 0.205). However, the evidence ratio reveals that the model with the F0min (model 2) was more than twice as good as the second best model (model 4). The addition of Dfmin or body size to the best model did not result in a better supported model (Table 1, comparing model 2 with model 5: LRT χ^2 = 1.82, d.f. = 1, p = 0.18; comparing model 3 with model 2: LRT χ^2 = 2.27, d.f. = 1, p = 0.13). The model which includes only body size as parameter was considerably less supported by the data (Table 1, model 13: < ΔAICc > 7). Thus, F0min was the factor more strongly correlated with dominance rank. Dfmin is also related to dominance rank, but to a lesser extent. Body size was not related to rank.

Table 1. Model selection to investigate the relationships between acoustic parameters and dominance rank and mating success in male fallow deer. The fit of the models is assessed by Akaike's information criterion (AICc): the lowest value indicates the best fit (in bold). K is the number of estimated parameters included in the model. ΔAICc gives the difference in AICc between each model and the best model. The Akaike's weights (w_i) assess the relative support that a given model has from the data, compared to other candidate models in the set. The evidence ratio is the ratio between the Akaike's weight of the best model and that of a competing one. This value is used to determine to what extent the best model is better than another. The covariates were: body size (=Body), dominance rank (Rank), minimum fundamental frequency (F0min), and minimum formant dispersion (DFmin).

Model		Log likelihood	K	AICc	ΔAICc	w_i	Evidence ratio
First set of models: Dominance rank							
1	Body	-3.86	3	16.12	5.82	0.024	18.33
2	F0min	**-0.95**	**3**	**10.30**	**0.00**	**0.443**	**1.00**
3	Body + F0min	0.19	4	12.07	1.77	0.183	2.43
4	Df	9.45	3	11.85	1.55	0.205	2.17
5	F0min + Df	8.08	4	12.52	2.22	0.146	3.04
Second set of models: Mating success							
6	Body	-11.41	3	31.22	8.16	0.012	59.22
7	Rank	**-7.33**	**3**	**23.06**	**0.00**	**0.682**	**1.00**
8	Body + Rank	-6.73	4	25.91	2.85	0.163	4.17
9	F0min	-11.15	3	30.70	7.64	0.015	45.64
10	Df	-10.95	3	30.31	7.25	0.018	37.53
11	F0min + Df	-10.48	4	33.40	10.34	0.004	176.27
12	Body + F0min	-9.55	4	31.54	8.48	0.010	69.69
13	Rank + Dfmin	-7.27	4	26.98	3.93	0.096	7.11

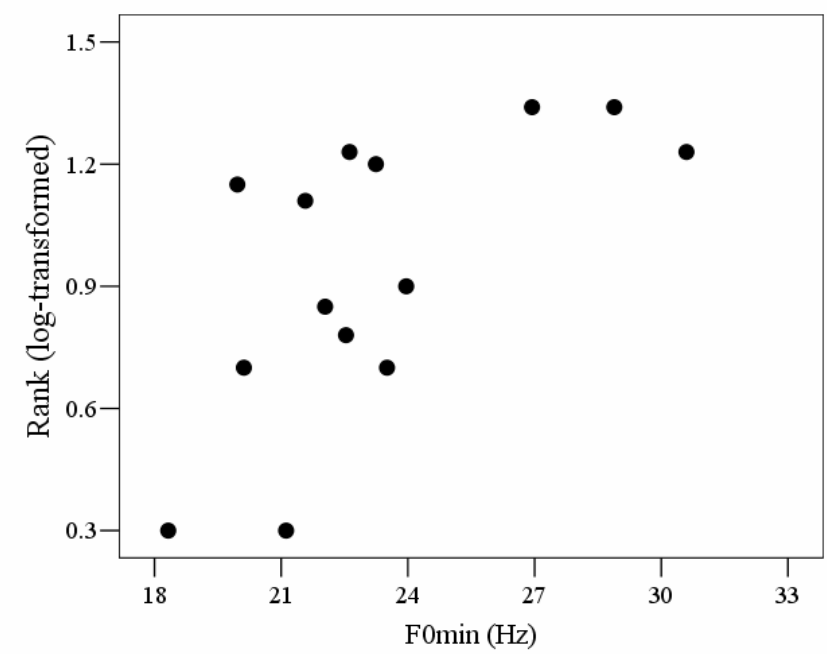

Fig. 3. The relationship between F0min and dominance rank (log-transformed), (n = 14 males, n = 156 groans). Lower values of dominance rank indicate higher ranking males.

Relationships between acoustic parameters and mating success

The model with only the rank included, was 61.9% supported by the data and clearly selected as the best model (lowest AICc; Table 1, model 7). This model was more than four times as good as the second best model in which body size was also included (model 8). The addition of body size did not significantly improve the best model (Table 1, comparing model 7 with model 8: LRT χ^2 = 1.19, d.f. = 1, p = 0.28). The models in which Dfmin, F0min were included together with rank, had considerably less support than the best model (Table 1, model 13: 3< ΔAICc < 7). All other models were poorly supported by the data (Table 1, model 6, 9, 10, 11, 12). Thus, dominance rank appears to be the crucial factor which determines male mating success in fallow deer, with higher-ranking males having higher mating success. Any relationship between the acoustics parameters and mating success appears to be mediated by rank.

DISCUSSION

We found that body size was negatively related to the minimum formant dispersion and not related to the fundamental frequency-related parameters of groans. We also found that minimum fundamental frequency and to a lesser extent, minimum formant dispersion, were related to dominance rank. Dominance in turn was strongly related to male mating success.

The acoustic structure of sexually selected calls often contains information on different phenotypic traits of the caller (Galeotti et al. 1997; Reby & McComb, 2003; Fischer et al. 2004). Recent research has shown that both fundamental frequency-related and formant-related parameters are important in determining the individuality of fallow deer groans (Vannoni & McElligott 2007). The results of this study suggest that the same acoustic parameters also have the potential to reliably signal male fitness-related traits, and the overall quality of the caller.

We showed that the fundamental frequency-related parameters of groans were not related to male body size in fallow deer. This is similar to results for other mammals (dogs, *Canis familiaris*, Riede & Fitch 1999; rhesus macaques, *Macaca mulatta*, Fitch 1997; red deer, Reby & McComb 2003; humans, *Homo sapiens*, Lass & Brown 1978, Van Dommelen 1993) and confirms that the growth of male larynx (and resulting fundamental frequency) is therefore at least partially dissociated from the growth of overall body size. In fallow deer, the fundamental frequency (F0) of calls is considerably lower in males than in females, both within adult and young age classes (Torriani et al. 2006; Vannoni & McElligott 2007; this study). Moreover, as fawns become older, the F0 of their calls decreases, at least during the first 3 months of life (Torriani et al. 2006). Overall these results show that in fallow deer, as well as in other sexually dimorphic mammal species, the F0 does not accurately indicate body size among adult males. However, its variation still reflects sexual size dimorphism in the vocal apparatus and may be used to distinguish sex and possibly stage of development of the animal.

Body size was strongly negatively related to the minimum formant dispersion. This result is of particular interest, considering that during vocalisation, males do not pull the larynx all the way down to its physiological limit (the sternum, McElligott et al. 2006). Therefore this casts doubt on whether formant frequencies still convey reliable information on male body size. During the breeding season, fallow deer males can groan more than 60 times per minute (McElligott & Hayden 1999, 2001). Previous research suggests that variation in short-term vocalisation rates transmit information mainly to other males, and that call rate could be used as an assessment cue in competitive interactions (McElligott & Hayden 1999). High call rates are in fact a function of energetic investment and can signal male condition (Prestwich 1994; Thomas 2002). However, similarly to red deer, conveying information about body size through formant dispersion might also be important in both

intersexual advertisement and mate attraction (Reby et al. 2005; Charlton et al. *in press*). Pulling down the larynx to an extent that is not maximum might therefore be adaptive for fallow bucks if this allows senders to reach high calling rate when a clear indication of body size is still provided.

Individual formants were related to body size to a lesser extent than the minimum formant dispersion (Dfmin). Higher formants (F5min and F6min) were more strongly related to body size than lower ones (F1min-F4min). These results agree clearly with those of studies conducted on red deer, dogs and primates, in which the relationships between formants and individual body size were attributed only to the higher formant frequencies and to the formant dispersion (Fitch 1997; Reby & McComb 2003; Rendall et al. 2005). Lower formants are thought to reflect vocal tract length less precisely than higher ones, but they may also be more difficult to measure accurately (Riede & Fitch 1999) and therefore show more variability. A recent study showed that higher formants are in fact more stable within individuals and suggest that they can be possibly used in individual recognition (Vannoni & McElligott 2007). Whatever the proximate mechanisms underlying the relatively low variability of higher formants (particularly of F5min and F6min), these results suggest that listeners could potentially use these cues to assess both the individual identity and size of the caller.

Males with higher ranks produced groans with lower minimum fundamental frequency (F0min). Our results are in contrast with those reported for other mammals such as baboons, in which higher-ranking males produce call with higher F0 (Fischer et al. 2004). High-ranking males are likely to be more motivated than low-ranking males, therefore producing calls characterised by increased sub glottal pressures which in turn determines increased F0. The same mechanism can also explain why red deer stags with higher reproductive success roar at higher fundamental frequencies (Reby & McComb 2003). In both baboons and red deer, F0 shows a high degree of inter-individual variability and is more likely to represent a cue to the motivational state or vocal effort of the animal rather than to physical characteristics of the caller. In contrast, F0 of fallow deer groans is relatively stable within individuals and accounts for a large proportion of vocal individuality (Vannoni & McElligott 2007). This discrepancy may originate from differential sexual selection pressure that might have favoured the evolution of vocal cues to the competitive ability in fallow deer males, and led to the selection of lower pitch vocalisations indicating higher-quality individuals.

The production of stable acoustic features that are limited by physiological constraints (such as the F0min), particularly in combination with physical activity, may indicate higher stamina and in general a higher Resource Holding Potential (RHP) of the caller (Fisher et al. 2004). Alternatively, the relationship between F0min and dominance status may be androgen-mediated and listeners could potentially use this acoustic cue to monitor the hormonal status of the males. This hypothesis is supported by substantial evidence of an association between androgens and vocal anatomy and between androgens and dominance in mammals (Beckford et al. 1985; Newman et al. 2000; Mazur & Booth 1997; Muller & Wrangham 2004; Beehner et al. 2006; Puts et al. 2006). However, studies investigating the direct influence of hormonal profiles on the acoustic characteristics of ungulate vocalisations remain to be carried out.

The minimum formant dispersion (Dfmin) was only marginally related to male dominance rank. This result is not surprising if we consider the lack of correlation between body size and dominance rank revealed by our analysis. The sample size we used in our study is probably too small to reveal an effect of body size on rank. In fact, the relationship between body size and dominance rank is not very strong even on a larger sample of males (McElligott et al. 2001). Assuming that a large body size is important for winning contests and therefore reaching high-ranking positions, males should use Dfmin as a cue to body size in order to assess the fighting ability of their opponents. This is indeed what has been recently shown in red deer in which males perceive the differences in the Dfmin of roars produced by different competitors and use them to adjust their vocalisations accordingly (Reby et al. 2005).

It is important to note that the majority of social dominance relationships between males are established through non-contact interactions before the rut and therefore before males became vocal. During this time, males live in bachelor herds, and direct assessment of body size and body mass of the competitors is likely to play the major role in determining the outcome of the interactions and therefore the dominance rank of the males (McElligott et al. 2001). During the rut, the dominance relationships previously established can be modified by fights. Because fights are expensive in terms of time, energy and risk of injury, males are expected to properly asses the actual status of their opponents before deciding to approach or avoid a competitor (Alvarez 1993; McElligott et al. 1998). Reliable assessment cues are those closely linked to the individual fighting ability and males may rely on several of these cues to assess each other both when they are distant and when they are close during the initial stage of the contest. The use of acoustic cues reflecting the individual RHP would then be crucial

especially when other cues, such as visual and olfactory cues, are not available, as for example at long distances and at night.

We found that dominance rank was the factor that was most strongly related to male mating success whereas body size appeared to play a minor (if any) role. This result confirms that in fallow deer, as in several other ungulate species, reaching a high ranking position is crucial for males to gain matings (Hogg 1987; McElligott et al. 2001; Saunders et al. 2005). However, in contrast with the results of a previous study investigating the relationship between body size and mating success in fallow deer (McElligott et al. 2001), we did not find a direct relationship between these two factors. Nevertheless, when we split our males (N = 14) into two groups, consisting of the seven most successful mating males and the seven least successful mating males, we found that the more successful males were significantly bigger than the less successful ones ($p = 0.03$).

We found that the acoustic parameters were not directly related to mating success. The relationships between the minimum fundamental frequency (F0min) and the minimum formant dispersion (Dfmin) with the mating success are therefore probably mediated by the dominance rank and body size of the males, respectively. In fallow deer, large investment in vocal display by males is critical to gaining matings (McElligott et al. 1999). Thus, while our data suggest that females may rely on acoustic cues such as Dfmin and F0min to identify bigger and higher-ranking individuals, other factors such as long-term investment in vocal display may be important for female to choose the best mate among those of similar quality.

Overall, our results are consistent with the hypothesis that male intrasexual competition was a salient selection pressure on the evolution of male vocal display in fallow deer. The ability to lower the larynx and therefore to produce groans characterised by lower formant frequencies probably evolved to allow senders to give receivers an exaggerated impression of body size, and therefore have advantages in intrasexual competition (Fitch 2002; Fitch & Reby 2001; McElligott et al. 2006). The production of low pitched vocalisations may have evolved because of the association between the fundamental frequency and phenotypic traits associated with the actual condition of the caller. By honestly signaling individual quality, males can properly evaluate each other and avoid costly and potentially dangerous fights. Moreover, if male vocalisations function as reliable signals of genetic quality, females may rely on these signals to choose among perspective mates

(Dawkins & Guilford 1996; Seyfarth & Cheney 2003). Playback experiments will be necessary to further investigate the independent influences of inter- and intrasexual selection on fallow deer groans.

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CHAPTER 6

Variation in the acoustic structure of groans and calling rate of fallow bucks during the rut

To be submitted

Variation in the acoustic structure of groans and calling rate of fallow bucks during the rut

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ABSTRACT

The acoustic structure of mammal vocalisations is affected by social and physiological factors and can therefore change over time. During the rut, fallow bucks reduce food consumption greatly, increase the time and energy in vocalising and fighting in order to gain matings, and this results in weight loss and poor condition. Therefore, we investigated if the acoustic structure of fallow buck groans and the groaning rate changed as a result of these factors . We found that F0-related parameters were lowest during the middle of the rut, and highest at the beginning and at the end. The measure F0 perturbation along the groan (Jitter) remains stable throughout the rut, whereas the number of pulses and duration of the groans decreased linearly. The minimum formant dispersion did not vary significantly with time. Groaning rate increased towards the peak of the rut when most mating occurred and then a rapidly decreased over the last days of the rut. Changes in the acoustics structure of groans and groaning rate represent changes in the intensity of intrasexual competition and mating opportunities occurring over the course of the rut, as well as the declining body condition of males.

INTRODUCTION

Recent research on mammal vocal communication has focused on determining the functions of calls by elucidating the information they convey to receivers (Fischer et al. 2002; Pfefferle et al. 2007; Theis et al. 2007). The information transmitted through vocalisations can be encoded in different aspects of a call, such as the acoustic structure of calls and the calling rate (McElligott & Hayden 1999; Manser 2001; Fischer et al. 2004). These vocal features may be affected by both physiological and social factors and therefore vary over both long and shorter-term timescales.

Changes in vocal behaviour have been shown to occur throughout the early ontogeny of many mammal species, including humans (Hammerschmidt et al. 2000; Scheiner et al. 2002; Pistorio et al. 2006; Torriani et al. 2006; Hollén & Manser 2007). Physical maturation during ontogeny leads to changes in the size or shape of the vocal apparatus, which directly affect the acoustic structure of vocalisations (Fitch & Hauser 1995; Fischer et al. 2002). Moreover, experience and learning may also be involved in individual vocal development (Seyfarth & Cheney 1986; Boughman & Moss 2003; Pistorio et al. 2006; Stoeger-Horwath et al. 2007). Ontogenetic vocal changes occur gradually over a relatively long-term timescale and are irreversible. In contrast, modifications to the acoustic structure of vocalisations, and other aspects (such as the calling rate) of individual vocal behaviour during adulthood are generally short-term reversible changes. These changes mainly occur in response to variation in the social context of an individual, which constitutes one of the most important features that influences vocal plasticity in adult mammals (Elowson & Snowdon 1994; Mitani & Brandt 1994; Gese & Ruff 1998; McElligott & Hayden 1999; Rukstalis et al. 2003).

Social context-related acoustic changes generally reflect changes in arousal or motivation of the animal. Intensity of arousal might be communicated in vocal signals by altered patterns of calling in space and time and/or by changes in the acoustic parameters of calls (Manser 2001; Fischer et al. 2002; Fitchel & Hammerschmidt 2002; Bachorowski & Owren 2003; Wich et al. 2003). For example, adult male baboons (*Papio cynocephalus ursinus*) emit the “wahoo” call at far greater rates during aggressive contests than comparatively low-arousal circumstances and the acoustic structure of these vocalisations changes following protracted calling (Fischer et al. 2002, 2004). Prolonged calling associated

with high physical activity has been shown to alter both the frequency pattern of calls as well as the calling rate (Liénard & Di Benedetto 1999; Fischer et al. 2004).

The physiological mechanisms at the basis of the different motivational states of the caller may largely contribute to the context-dependent acoustic modifications (Morton 1977, 1982). Short-term variation in the arousal state may alter respiration and therefore vocal production (Titze 1994; Scherer 2003). Moreover, fluctuations in hormone release occurring during social interactions may influence both anatomical and neurophysiological structures involved in the production of vocal signals and therefore result in modified acoustic patterns (Dabbs & Mallinger 1999; Semple & McComb 2000; Yamaguchi & Kelley 2002; Manteuffel et al. 2004; Yeon et al. 2006).

In order to understand the evolution of any vocal signal, it is important to explore in detail the source of its variation by investigating short-term changes in both the acoustic structure of calls and other aspects of vocal behaviour (e.g. calling rate), which might indicate different communicative functions. Modification of the acoustic structure of the call within individuals over time may have implication for individual recognition, whereas changes in the calling rate may provide information on the current motivation and condition of the caller (Jorgensen & French 1998; Rukstalis et al. 2003; Theis et al. 2007).

Sexually selected calls produced by fallow deer males provide an excellent opportunity to investigate short-term vocal changes in mammals. Fallow bucks, as well as males of other polygynous deer, vocalise only in two specific contexts during their lives. When they are fawns, they emit naturally-selected contact calls, and the acoustic structure of these calls undergoes ontogenetic changes (Torriani et al. 2006). Males then remain largely silent until they are old enough to compete with other males (McElligott et al. 1999). When they become socially mature (> 4 years old), males do not vocalise for most of the year, and then have an extremely intense period of vocal activity during the rut. At this time they produce a repetitive and stereotypic call known as groan (Reby et al. 1998; Vannoni & McElligott 2007). Two types of groans can be identified; the common and the harsh groan (Vannoni & McElligott 2007). Harsh groans are generally only produced during intense chasing and herding behaviour, whereas common groans are given in all contexts (Vannoni & McElligott 2007). The acoustic structure of fallow deer groans is individually distinctive (Reby et al. 1998; Vannoni & McElligott 2007) and contains information on male quality

(Reby & McComb 2003; McElligott et al. 2006; Vannoni & McElligott *submitted*). Males start groaning several weeks before the first matings take place, and they maintain high groaning rates over prolonged periods (McElligott & Hayden 1999; McElligott et al. 1999).

Variation in the number of nearby competing males and oestrous females during the rut determine short-term changes in arousal and motivation of the males and these affect their vocal behaviour (McElligott & Hayden 1999). Furthermore, the decline in body condition that males undergo at this time (McElligott et al. 2003) may also affect both physiological and behavioural mechanisms, including vocal display. Male vocal activity is generally reduced over the last days of the rut (E. Vannoni & A.G. McElligott pers. obs.). Moreover, groans given at the end of the rut usually sound noisier and higher in pitch than those given at the beginning (E. Vannoni & A.G. McElligott pers. obs.). Changes in vocal behaviour during the rut may therefore be used as a potential source of information for competing males that could be monitoring the motivation and condition of their opponents. Fights between males that differ widely in terms of dominance rank are infrequent early in the rut, but become more frequent towards the end (McElligott et al. 1998). Long-term investment in male vocal display may influence female mate choice (McElligott et al. 1999). However, females may also monitor male current condition and use this information to evaluate perspective mates (Komers et al. 1999). There is already some evidence for deer that males and females are able to perceive relatively small changes in formant frequencies and use them for assessment in both male-male competition and female choice (Reby et al. 2005; Charlton et al. 2007; Charlton et al. *in press*).

In this study, we investigated if changes in the acoustic structure of groans and groaning rate occurred over the course of the rut. We first extracted and measured the acoustic parameters of groans recorded at multiple time points during the rut. We then we determined whether the time of the rut has an effect on both the acoustic structure of groans and the groaning rate.

METHODS

Study site and population

The study was conducted on a herd of European fallow deer in Phoenix Park (709 ha, 80% pasture, 20% woodland; 53° 22' N, 6° 21' W), Dublin, Ireland. All males used in this study were of known age, tagged and were therefore individually recognisable.

Recording and selection of groans

Recordings were made using a Sennheiser MKH 70 directional microphone connected to a Sony digital audio tape recorder DAT-TCD D100. Groans were recorded between dawn and sunset, at a distance of 10 to 50 m from the vocalizing animal.

Vocalizations were imported into a computer using Avisoft-SASLab Pro 4.38 at a sampling rate of 22.05 KHz and saved in WAV format, and at 16-bit amplitude resolution (Vannoni & McElligott 2007, Vannoni et al. 2005). The recordings that did not contain energy above 8 KHz were down-sampled to 16 KHz for a better frequency resolution. Narrow-band spectrograms of groans (FFT method, window length = 0.03 s, time step = 1000, frequency step = 250, frequency resolution = 20 Hz, Gaussian window shape, dynamic range = 35 dB) were edited using Praat 4.5.01 DSP package (P. Boersma & D. Weenink, University of Amsterdam, The Netherlands). Only common groans with a low level of background noise were considered for analysis.

Recordings were carried out during three consecutive ruts (2002, 2003, and 2004). In previous research, the "rut" was used as the period between the days on which the first and last mating occurred (mid October to early November; McElligott et al. 1999). Good quality recordings were available from October 8 to 31. This period represents the time during which most of the males start vocalising (McElligott et al. 1999) and because it includes only a few days before the first mating occur, we decided in this study to refer to it as rut. Our hypothesis concerned whether the acoustic characteristics of groans and the groaning rate change during the rut. We therefore only considered males for which we had good recordings over a time period of at least 6 days throughout this period. We included in our analysis males between 5 and 8 years old because they spend the highest proportion of time vocalising (McElligott et al. 1999). Moreover, they had reached their asymptotic size and were not undergoing changes associated with senescence (McElligott et al. 2002).

Sound analysis

Groans are low-pitched vocalizations and are generally characterized by a pulse-train structure (Fig. 1). The pulses represent the vibrations of the vocal folds and determine the fundamental frequency (F0) of the call. Fundamental frequency is defined as the inverse of the interpulse interval and this can be measured as the distance between consecutive pulse onsets. Distances between pulses were measured automatically from the envelope (amplitude vs. time) of the signal by using Pulse Train Analysis in Avisoft-SASLab Pro 4.38 (Vannoni & McElligott 2007). We calculated the values of the F0 along the groan and then averaged these values to obtain the mean F0 (F0mean). Because the fundamental frequency varies over the duration of the groan, the minimum and the maximum F0 (F0min and F0max) were also included in the analysis. From the envelope of the signal, we also calculated the number of pulses (Pulses) and the duration of the groan (Duration) as the distance between the onset of the first pulse and the end of the last one. Finally, we quantified the variation in F0 along the call using a measure of F0 perturbation, known as Jitter (Titze 1994). Jitter has been used as a measure of voice quality in mammals (Rendall 2003) and it has been shown to vary under prolonged vocal effort in humans (Rantala et al. 1999; Laukkanen & Kankare 2006). In this analysis, Jitter was calculated by dividing the average absolute difference between consecutive frequencies, by the mean F0 per groan (Peak-picking method, Horii 1979; Titze et al. 1987).

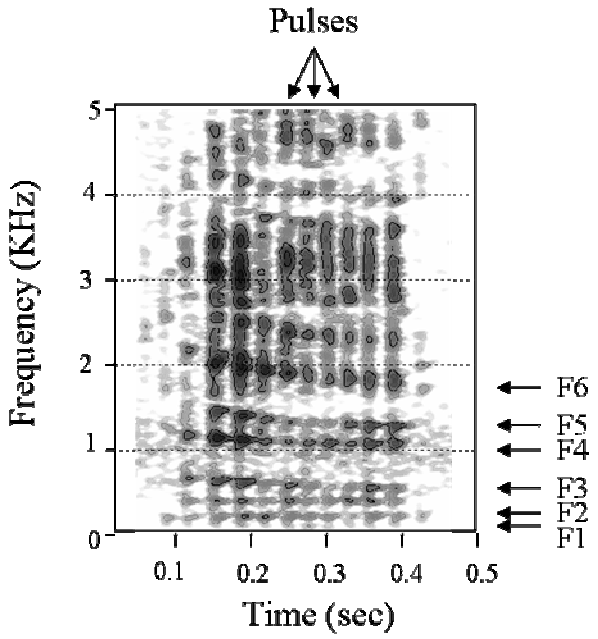

Figure 1. Narrow band spectrogram of a groan. On the spectrogram, the pulses and the first six formants are indicated.

Groans contain six formants within the first 2.5KHz and these are evident as horizontal frequency bands in the spectrogram (F1-F6 in Fig. 1). The formant frequency decreases along the groan reflect the elongation of the vocal tract occurring during vocalisation (McElligott et al. 2006). Formants were estimated using Linear Predictive Coding analysis (LPC), (Sound: To Formant (burg) command) in Praat 4.5.01 DSP package (Vannoni & McElligott 2007). We calculated the minimum frequencies of the six formants (F1min-F6min) from each groan by averaging the values over the last part of the call when formants become flat. This is the time when the larynx is pulled down to the maximum extent (McElligott et al. 2006). From the values of the minimum formant frequencies, we estimated the minimum spacing of the formants (known as minimum formant dispersion, (Dfmin), according to Reby & McComb (2003). Only this last parameter was included in the statistical analysis as it has been shown to vary with increasing motivation in deer (Reby et al. 2005).

It was not always possible to measure the fundamental frequency and formants of the same groans for all males because of variation in the recording conditions. In addition, the groaning activity (both in terms of number of vocal males and time spent vocalising) changes during the rut (McElligott et al. 1999). As a result, sample sizes (number of groans and number of males) varied among analyses (Table 1). In total, we analyzed recordings of 20 different males (6 in 2002, 1 in 2003, and 13 in 2004). For the same males, we also calculated the groaning rate per minute, by dividing the number of groans of each recording sequence by the duration of the sequence. Data on the F0-related and temporal parameters were available from October 9-31 (except on October 30). Data on the formant frequencies were available from October 9-28 (except on October 14). Data on groaning frequency were available from October 8-31.

For descriptive purposes we calculated values of the acoustic parameters and of the groaning rate in time-periods across the rut. We grouped the days of the rut in four time-periods based on the distribution of the observed matings (and therefore the mating opportunities) during this time, which is quite consistent across years (AG McElligott, unpublished data; Fig. 2). Because the number of matings is not constant across the rut, the four time-periods are different in length. The first time-period (pre-mating rut, 7 days) includes those days during which matings do not usually occur or occur very rarely. During the second time-period (early rut, 6 days), the number of matings increases but overall only a small proportion (15% or less) of the total number of matings had usually occurred. The third time-period (middle rut, 5 days) represents the peak of matings and the fourth time-period

(late rut, 6 days) includes the days from the peak to the end of the rut, during which the number of matings declines drastically.

Table 1. Number of males and groans (Mean and SE) analyzed for each day of the rut.

Day (October)	F0-related and temporal parameters			Formant dispersion		
	Males	Groans		Males	Groans	
		Mean	SE		Mean	SE
8	-	-	-	1	5.0	-
9	3	10.7	2.4	2	5.5	0.5
10	1	5.0	-	2	5.0	0.0
11	3	10.7	1.8	3	8.0	1.0
12	2	5.0	0.0	3	8.0	1.7
13	1	13	-	1	10.0	-
14	3	9.3	2.6	-	-	-
15	5	5.8	1.1	2	7.5	0.5
16	3	13.0	2.6	3	8.7	1.3
17	4	4.8	0.5	3	6.3	0.7
18	7	13.0	2.1	3	6.7	1.8
19	10	9.2	1.6	7	6.3	0.6
20	9	8.4	2.0	7	9.0	2.3
21	2	16.5	4.5	1	6.0	-
22	6	10.0	2.2	4	7.5	1.8
23	7	10.6	2.2	1	8.0	-
24	9	12.9	2.3	6	7.7	0.9
25	2	13.5	5.5	1	10.0	-
26	13	10.5	1.6	5	7.2	1.6
27	6	9.7	2.1	5	7.2	0.8
28	4	14.8	5.1	2	5.5	0.5
29	3	15.7	5.3	-	-	-
30	-	-	-	-	-	-
31	1	19.0	-	-	-	-

F0-related and temporal parameters were measured on groans of 20 different males while formant dispersion was measured on groans of 12 different males.

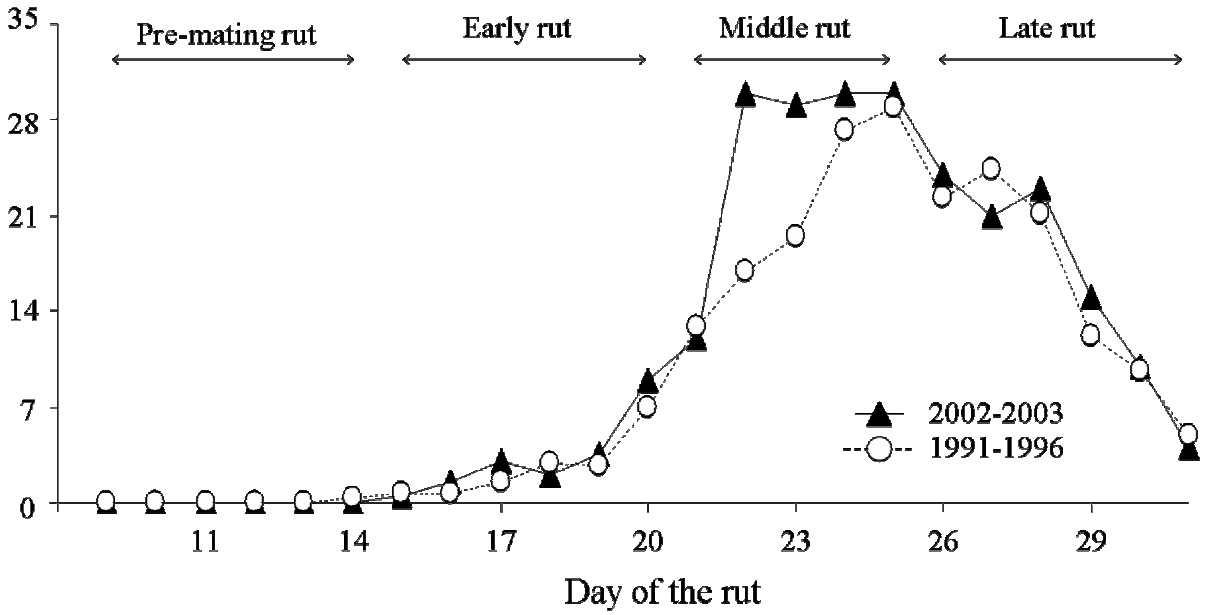

Figure 2. Distribution of matings throughout the rut for the years 1991-1996 and 2002-2003. The four time-periods (Pre-mating rut, Early rut, Middle rut, and Late rut) used for descriptive statistics are indicated.

Statistical analysis

We used a general linear mixed effect model (GLMM) procedure fitted with residual maximum likelihood estimation (REML, lme function; Venables & Ripley 2002) to investigate the effect of day of the rut on the acoustic parameters of groans (F0-related parameters: 1107 groans; 55.3 ± 4.7 per individual, N = 20; Formant dispersion: 454 groans; 37.8 ± 5.0 per individual; N = 12) and on the groaning rate (Groaning rate: 235 sequences; 11.7 ± 1.2 per individual; N = 20). The acoustic parameters of each analyzed groan as well as groaning rates were averaged within each male for each day of the rut. Individual identity nested within year of recording was fitted as a random term. In this way, we controlled for repeated measurements of the same individual during the rut and for pooling data from different years of recording. Because both linear and curvilinear relationships were possible between the acoustic parameters and the days of the rut, both a linear and quadratic terms were fitted as fixed effects. The calling rate of deer is known to increase from the beginning to the peak of the rut and then to decrease towards the end (Clutton-Brock & Albon 1979; McElligott & Hayden 1999). We therefore fitted only the quadratic term as fixed effect to the model involving the groaning rate. Normal distributions of the data were determined by visually inspecting Q-Q plots and scatter plots of the residuals of the dependent variables. All tests were 2-tailed and factors were considered to have a statistically significant influence if $p < 0.05$. All means are given with standard errors.

RESULTS

Acoustic parameters of groans

Descriptive statistics of the acoustic parameters over the course of the rut are given in Table 2. The average day-to-day change was 4.5 ± 0.5% (N = 21; range = 0.1-8.4%) for F0mean, 2.8 ± 0.4% (N = 21; range = 0.3-7.7%) for F0min, 6.6 ± 0.9% (N = 21; range: 0.9-13.7%) for F0max, and 15.9 ± 2.6% (N = 21; range: 1.1-36.7%) for Jitter. The number of pulses (Pulses) and the duration of the groan (Duration) varied on average from one day to the next, 6.9 ± 1.3% (N = 21; range: 0.1-20.1%) and 7.2 ± 1.0% (N = 21; range: 0.1-20.8%), respectively. The average day-to-day variation for F1min was 7.3 ± 1.1% (N = 19; range: 1.9-19.2%), for F2min, 2.0 ± 0.4% (N = 19; range: 0.2-7.1%), for F3min, 5.1 ± 0.8% (N = 19; range: 0.5-12.0%), for F4min, 1.7 ± 0.3% (N = 19; range: 0.0-4.7%), for F5min, 1.2 ± 0.3% (N = 19; range: 0.0-4.5%), for F6min, 1.5 ± 0.3% (N = 19; range: 0.3-4.3%), and for Dfmin was 1.2 ± 0.2% (N = 19; range: 0.3-3%).

F0min and F0mean varied significantly over the course of the rut (Table 3). In the regression models, both the linear and the quadratic terms were significant for F0min, whereas only the quadratic term was significant for F0mean (Table 3). F0min and F0mean were higher at the beginning and end of the rut, than during the middle (Fig. 3a, b). The direction of changes of F0max and Jitter during the rut was similar to that of the other F0-related parameters. These changes were marginally significant for F0max whereas for Jitter they were not significant (Table 3). Pulses and Duration showed a significant linear decrease as the rut progresses (Table 3, Fig. 3c, d). The minimum formant dispersion did not significantly change (Table 3).

Table 2. Descriptive statistics for the acoustic parameters of groans and groaning rate for each time-period (Pre-mating rut, Early rut, Middle rut, and Late rut) of the rut.

	Pre-mating rut (8-14 October)		Early rut (15-20 October)		Middle rut (21-25 October)		Late rut (26-31 October)	
Acoustic parameters	Mean	SE	Mean	SE	Mean	SE	Mean	SE
F0-related								
F0mean (Hz)	28.09	0.85	26.96	0.44	25.69	0.51	27.51	0.45
F0min (Hz)	22.53	0.49	21.22	0.11	21.25	0.36	22.37	0.27
F0max (Hz)	34.28	1.33	33.25	0.99	31.08	0.84	33.50	0.88
Jitter (Hz)	13.48	0.92	13.16	0.87	12.66	0.32	14.08	0.89
Temporal								
Pulses	10.21	0.25	9.87	0.13	8.93	0.21	8.89	0.41
Duration (ms)	351.24	6.16	353.85	7.73	330.66	10.41	307.03	11.11
Formant-related								
F1min (Hz)	195.32	3.64	207.35	3.93	204.56	4.76	185.90	6.04
F2min (Hz)	413.54	1.21	414.26	5.27	402.23	2.97	407.34	3.92
F3min (Hz)	553.94	9.17	568.32	9.58	576.53	10.62	565.671	13.53
F4min (Hz)	1060.82	6.49	1058.12	3.72	1032.79	6.74	1076.43	1.55
F5min (Hz)	1258.25	4.75	1271.48	8.03	1252.60	8.38	1283.36	6.75
F6min (Hz)	1814.04	12.16	1787.22	4.13	1748.03	16.46	1803.20	8.80
Dfmin (Hz)	300.07	1.36	299.31	1.32	293.89	2.06	301.81	1.44
Groaning rate (groans/min)	23.89	3.09	32.81	1.98	44.73	1.10	32.46	2.63

Table 3. General linear mixed effect models to investigate the effect of day of the rut on the acoustic parameters of groans and groaning rate. Both the linear (Day of rut) and the quadratic term (Day of rut^2) were fitted as fixed effects.

		Day of rut		Day of rut^2	
Acoustic parameters	df	F	p	F	p
F0-related					
F0mean (Hz)	83	0.00	0.98	8.28	0.005
F0min (Hz)	83	9.12	0.003	12.03	< 0.001
F0max (Hz)	83	0.48	0.49	3.45	0.07
Jitter (Hz)	83	0.002	0.97	0.49	0.49
Temporal					
Pulses	83	24.13	< 0.001	0.04	0.85
Duration (ms)	83	25.77	< 0.001	3.96	0.05
Formant-related					
Dfmin (Hz)	48	0.23	0.64	0.17	0.69
Rate (groans/min)	110	52.15	< 0.001	24.13	< 0.001

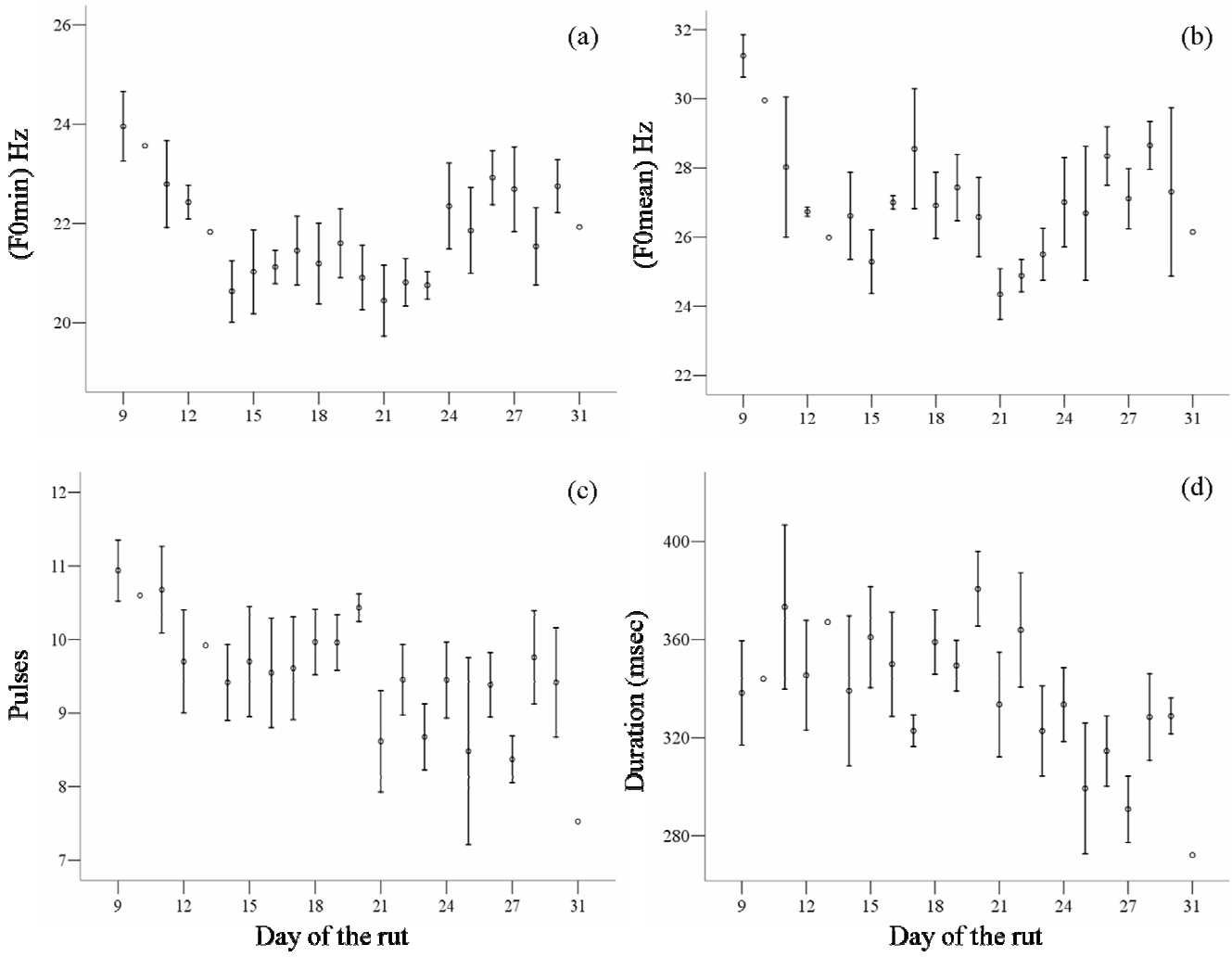

Figure 3. F0min (a), F0mean (b), number of pulses (c), and duration of the groan (d) over the course of the rut. The mean of each acoustic parameters among the males was calculated. The error bars show the standard errors of the means. Data on the F0-related and temporal parameters were available from October 9-31 (except October 30).

Groaning rate

Groaning rate was calculated for 5.54 ± 0.55 (Range 1-12) different males for each day of the rut. We used 1.75 ± 0.12 (range 1-8) recording sequences (sequence length: 0.78 ± 0.38 min) for each male in every single day. Descriptive statistics of the groaning rates during the rut are given in Table 2. The average day-to-day variation was 21.5 ± 4.1% (N = 24; range = 0.5-71.3%). Groaning rate varied significantly over the course of the rut (Table 3), increasing towards the peak of the rut and then decreasing until the end (Fig. 4).

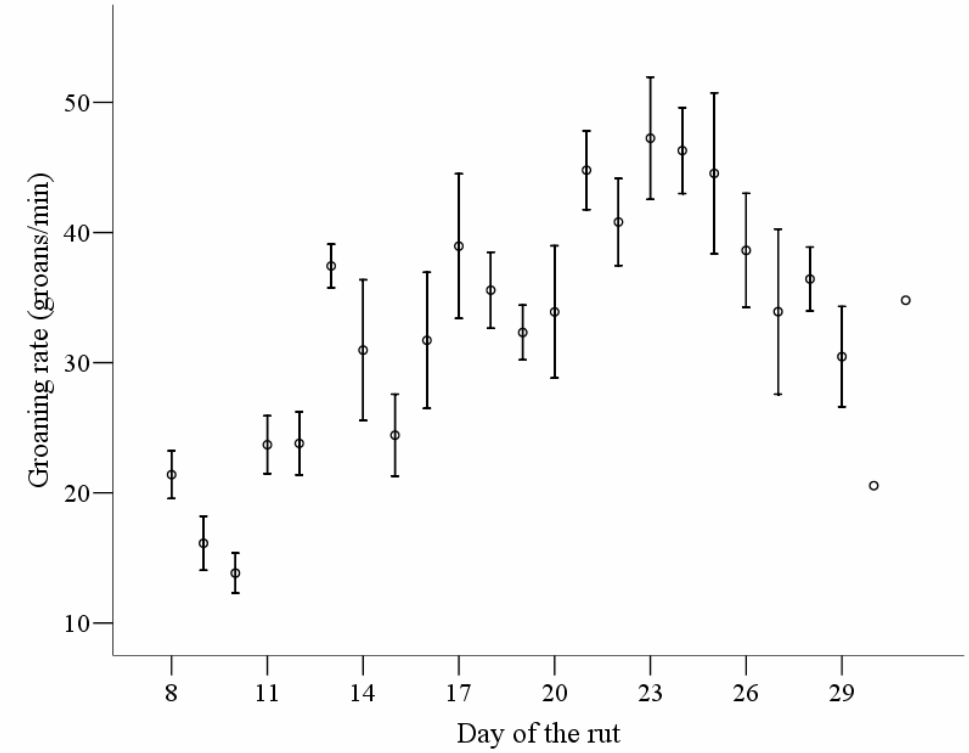

Figure 4. groaning rates (groans/min) during the course of the rut. The mean of the groaning rate among the males was calculated. The error bars show the standard errors of the means. Data on groaning rate were available from October 8-31.

DISCUSSION

We found that the acoustic structure of fallow deer groans and the groaning rate changed over the course of the rut. The mean and the minimum fundamental frequency (F0mean and F0min) were lowest during the middle of the rut and highest at the beginning and at the end, whereas the temporal parameters (number of pulses and duration of the groans) decreased

linearly with time. By contrast, the minimum formant dispersion (Dfmin) showed minor changes and therefore remained relatively stable during the rut. Groaning rate rapidly increased from the beginning to the peak and then decreased over the last days of the rut. Previous research has shown that the acoustic characteristics of sexually selected calls are individually distinctive and related to the body size of the caller which is generally fixed among adult individuals (Fischer et al. 2002,2004; Reby & McComb 2003; Sanvito et al. 2007; Vannoni & McElligott 2007; Vannoni & McElligott *submitted*). This suggests that the acoustic structure of these calls is rather stable over time. Our results show that the acoustic structure of calls and the calling rates change over a short period of a few weeks. This probably results from variation in traits related to the current condition of the caller, as well as changes in the availability of mating opportunities and the intensity of male-male competition.

Fallow deer groans are individually distinctive with F0min and the minimum frequency of the higher formants (F4min-F6min) contributing most to the inter-individual variation (Vannoni & McElligott 2007). The alteration of acoustic signals that contain individual signature information represents a potential obstacle for individual recognition of vocalisations. In the absence of acoustic stable characteristics, listeners may still be able to perceive individual differences in calls if the neural basis of recognition system can tolerate a wide range of variation in call structure, and if the changes are within the tolerance range. In humans, voice individuality is retained over a wide range of pitch range, especially towards the low frequency region (Kuwabara & Takagi 1991). By contrast, the perception of voice individuality is significantly affected by changes in formant frequencies and is seriously compromised by a formant shift of approximately 5% of the original formants (Kuwabara & Takagi 1991).

Mammals can recognise each other based on acoustic cues and are able to perceive differences in the acoustic parameters of calls related to variation in size, age and other phenotypic attributes of the caller (Sayigh et al. 1998; Weiss et al. 2001; Kitchen et al. 2005; Reby et al. 2005; Charlton et al. *in press*). However, experiments investigating how smaller vocal changes affect individuality in species other than humans have not been carried out. In red deer, males and females can perceive a shift in formant frequencies of about 10% (Reby et al. 2005; Charlton et al. 2007, *in press*) and variation in formants across the period of vocal activity has been shown to decrease individuality in the common roars (Reby et al. 2006). Our

results suggest that the degree of variation showed by F0min and F4min-F6min during the rut should ensure the individually distinctive characteristics of fallow deer groans. Playback experiments with modified calls are necessary to test this hypothesis.

It is important to note that F0min plays a bigger role in determining individuality in fallow deer groans than in calls of other species (Vannoni & McElligott 2007). Therefore, it is possible that fallow deer possess better pitch discrimination perceptual abilities than other animals and that even very small variation in F0min may alter individual recognition. In this case, individuals must have the capacities to recognise, modify, and assess the stored representation of a voice as it changes. The neural mechanism which would allow animals to develop such capacities has been shown to be present in mammals as well as the ability to memorise different versions of individual calls (Wang 2000; Charrier et al. 2003).

We found that F0min changes followed a curvilinear trend over the course of the rut. F0min showed a decline during the first days of the rut and then remained relatively stable until the peak. A previous study showed a negative relationship between the F0min of groans and male dominance rank (Vannoni & McElligott *submitted*), suggesting that lower pitch may indicate higher male quality. The increase in both physical and vocal activity during the first part of the rut may force males to vocalise at the upper limits of stable vocal fold vibration. The production of low-pitch calls at a high vocalisation rate may significantly increase the effort and control required to maintain the performance of the vocal production. Calls indicating a stable and regular vibration of the vocal folds may therefore signal better physical condition of the caller (Riede et al. 2007). This would be particularly important during the days that precede the peak of the rut when most of the females approach oestrus and are therefore likely to choose among perspective males (Komers et al. 1999). In red deer females do not prefer low-pitched roars (McComb 1991) and the fundamental frequency is though to transmit information mainly on the motivational state of the caller (Reby & McComb 2003). However, there is some evidence that F0min is negatively related with dominance in fallow deer (Vannoni & McElligott *submitted*) and this may have driven female preferences towards low pitched sounds as it has already been proposed for humans (Feinberg et al. 2005; Puts et al. 2006).

At the peak of the rut, F0min increased and stayed at a high level until the end of the rut. The degree of energetic expenditure due to conspicuous displays occurring at this time is

very high, especially if the loss in weight that males undergo during the rut is considered (McElligott et al. 2003). If the production of groans with low F0min communicates better physical condition, then it would always be advantageous for males to produce these groans. However, affording such costs might not be possible anymore from the peak of the rut onwards because of physical exhaustion. This, together with the physiological and biomechanical mechanisms at the basis of acoustic alterations due to prolonged vocal effort, may lead to pitch perturbations and a consequent increase in F0 (Welham & Maclagan 2003).

Changes occurring in F0mean and F0max went in the same direction as those of F0min with values decreasing from the beginning to the peak of the rut and increasing during the late rut. These changes were significant for F0mean and only marginally significant for F0max. In general, the profiles followed by F0mean and F0max changes are less clear due to a higher inter-individual variation. This is also probably the reason why the relatively larger frequency changes in Jitter do not follow a specific distribution during the rut. Variation in F0mean, F0max and Jitter may therefore have the potential to reveal the effects of momentary emotional states as they occur (Soltis et al. 2005; Theis et al. 2007). The analysis of groans recorded before, during and after a fight would be necessary to test this hypothesis.

We found that the minimum formant dispersion (Dfmin) did not change significantly during the rut. Single formants (especially the lower ones) may vary because the articulatory movements and therefore the vocal tract shape changes with different vocalisations (Rendall et al. 1998). However, this does not affect the overall formant dispersion. Males are therefore able to pull down the larynx always to the same level despite changes in motivation, physical activity, and groaning rate (Vannoni & McElligott 2007). Formants frequencies have been shown to be less sensitive to vocal effort than fundamental frequency (Liénard & Di Benedetto 1999). Additionally, acoustic alteration due to changes in motivation mainly affect the activity of the larynx and not of that of the vocal tract. A stable Dfmin through the rut allows the information about body size of the males to be always reliably conveyed.

The number of pulses and the duration of the groans gradually decreased as the rut progressed. Longer calls are usually produced at slower rates, thereby keeping aerobic metabolic costs relatively constant (Wells & Taigen 1986; Kajikawa & Hasegawa 2000). However, as changes in groaning rate and temporal parameters followed a different distribution (curvilinear and linear respectively) over time, this seems to be not the case in our

study. Fallow deer males lose an average of 26% of their body mass during the rut (McElligott et al. 2003). This decline in body condition, due to a dramatic reduction in forage intake and increased energy expenditure, is likely to occur gradually and this is reflected in the linear reduction in the number of pulses, and consequently in the duration of the groans.

The changes in the groaning rate during the rut are more conspicuous than those found in the acoustic structure of groans. Previous research has shown that the groaning rate varies according to different social contexts and suggests that short-term changes in groaning rate mainly represent a threat signal directed to other males (McElligott & Hayden 1999; McElligott and Hayden 2001). In our study, we did not take into consideration the specific social context of groan emission. Nevertheless, variation of the groaning rate during the rut followed a very clear curvilinear trend, reaching the highest value at the peak of the rut when most matings occurred, and being lower during the early and late rut. This suggests that groaning rate might change according to changes in motivation caused by variation in the number of females available for mating. The presence of more females in oestrus and rivals attempting to gain access to them, may determine of a higher level of arousal in the caller. This may result in change in breathing rate and therefore in the groaning rate (Scherer 2003). The rapid decrease in groaning rate at the end of the rut may also be partly explained by the decline of male body condition (see also Clutton-Brock & Albon 1979).

It is important to note that the males included in this study are among those that vocalised through most of the rut. These males lose a great deal of condition during this period, start producing hoarse groans late in the rut, and also get challenged by formerly subordinate males (E. Vannoni & A.G. McElligott pers. obs; McElligott et al. 1998). Many of the prime-aged males stop vocalising and also their efforts to gain matings, and return to the area they occupy for the rest of the year (E. Vannoni & A.G. McElligott pers. obs). A large energy investment in term of vocal display is characteristic of high-quality males (McElligott et al. 1999). It is therefore likely that we recorded and analysed only very successful males (high dominance rank and high mating success).

Our results show that the acoustic structure of fallow deer groans and the groaning rate change over the course of the rut and suggest that these acoustic features have different communicative functions. Groaning may therefore represent a unimodal multicomponent signal by providing information about different aspects of male quality (Candolin 2003). In

particular, acoustic components that are stable over time, such as the formant dispersion, contain information about fixed characteristics of the caller (e.g. body size) generally related to the genetic quality of the males. By contrast, dynamic acoustic features may convey information about aspects of the current body condition of the caller. The interaction of different quality-indicator components may improve information reliability and the discrimination of male quality (Rowe 1999). Further investigation involving physiological measurements and playback experiments are needed to determine the extent to which acoustic variation can be attributed to the modification of the individual physiological state and if this variation can be perceived and used by conspecifics.

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This is the end
Beautiful friend
This is the end
My only friend, the end
(The Doors)

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PUBLICATIONS

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